

***Commercial Vegetative Inoculum of  
Pisolithus tinctorius and Inoculation  
Techniques for Development of  
Ectomycorrhizae on Bare-root  
Tree Seedlings***

BY

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**ABSTRACT.** Vegetative inoculum of *Pisolithus tinctorius* (Pt) produced by research procedures at the Institute for Mycorrhizal Research and Development (IMRD) was compared with that produced in various commercial solid substrate fermentors by Abbott Laboratories. Effectiveness of inocula was examined during 1977 through 1980 in 54 discrete experiments undertaken at the IMRD Microplot Nursery and in 33 conventional bare-root nurseries located in 25 states. Over 750,000 seedlings of 11 pine species and varieties, Douglas-fir, and northern red oak were involved. Inoculum was broadcast on fumigated nursery soil at different rates and manually mixed into the upper 10 cm of soil before sowing seed. In conventional nurseries, most Abbott inoculum batches produced in 1977 and 1978 were ineffective, but modifications in fermentation procedures significantly improved effectiveness in 1979 and 1980 to such a degree that most batches of Abbott inoculum were as effective as IMRD inoculum. The minimum standard for inoculum effectiveness was formation by Pt of at least 50 percent of all ectomycorrhizae on inoculated seedlings. In the 1980 tests, a single drench application of captan (5.6 kg a.i./ha) after sowing of seed significantly improved inoculum effectiveness.

A nutrient-enriched medium of vermiculite and peat moss, the latter at 5 to 10 percent by volume, was best for growing Abbott inoculum. Peat moss maintained the pH of inoculum below 6.0 and increased efficacy. Inoculum leached with water and dried was most effective. Of seven inoculum characteristics studied, no one was consistently correlated with effectiveness of inoculum in nursery tests. Generally the most effective inoculum was one with (1) abundant hyphae of Pt inside the vermiculite particles, (2) pH between 4.5 and 6.0, (3) minimum microbial contaminants, and (4) water soluble substances such as glucose minimized by leaching before drying. An inoculum broadcast rate of 1.08 l/m<sup>2</sup> of soil surface gave best results.

The product of percent of seedlings ectomycorrhizal with Pt and proportion of Pt ectomycorrhizae to total ectomycorrhizae on inoculated seedlings was termed the Pt index. Attempts were

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made to correlate final Pt indices formed by IMRD inoculum on 1-0 loblolly pine seedlings in 20 nursery experiments with various cultural practices and soil conditions in order to determine the effects of these individual factors on inoculum effectiveness. No single factor accounted for significant differences in inoculum effectiveness even though each factor varied from nursery to nursery.

Loblolly pine seedlings from 11 conventional nursery tests in which Pt indices were >50 produced 35 percent more biomass/cm<sup>2</sup> of nursery soil than did seedlings with natural ectomycorrhizae. This suggested that seedlings with more than half of their ectomycorrhizae formed by Pt utilized water and nutrients more effectively and that, in most nurseries, successful inoculation with Pt could lead to more plantable seedlings/unit area of nursery soil than could otherwise be produced with the same cultural practices.

In the 1-0 pine nurseries, Pt indices above 50 were associated with larger seedlings and fewer culls in comparison to noninoculated control seedlings in over half of the nurseries. In most of the remaining nurseries, however, control seedlings had abundant natural ectomycorrhizae, grew well, and included few culls.

In the 2-0 seedling nurseries, only 7 of 12 tests had Pt indices >50 after the first growing season and only two had Pt indices >50 at the end of the second growing season. Significant seedling growth increases and a corresponding reduction in seedling culls due to Pt ectomycorrhizae were found in only 4 of these 12 nurseries. Results from one 3-0 pine seedling test in Michigan were similar to those of the 2-0 tests.

There were indications that soil fumigation in the fall was not as effective as spring fumigation for development of Pt ectomycorrhizae. Only 30 percent of the nurseries in the 1978 test that fumigated soil in the fall of 1977 preceding spring inoculations had positive inoculation results, whereas 80 percent of the nurseries that fumigated soil in the spring of 1978 had positive results.

The number of fruit bodies produced by Pt in the various nurseries was positively correlated with high Pt indices. *Thelephora terrestris* was the most frequently encountered naturally occurring ectomycorrhizal fungus in these tests. In 41 of the 45 tests in conventional nurseries, fruit bodies of *T. terrestris* were found on all tree species in 23 of the 25 states in which tests were undertaken. Fruit bodies of *Rhizopogon nigrescens* and *Laccaria laccata*, and the jet-black ectomycorrhizae formed by *Cenococcum geophilum* were rarely encountered.

Results of these tests showed that viable vegetative inoculum of Pt can be effectively produced by industrial fermentation procedures and used to form ectomycorrhizae in bare-root seedlings of a variety of forest tree species for practical use in forestry.

**ADDITIONAL KEY WORDS.** *Pinus taeda*, *P. elliotii* var. *elliottii*, *P. echinata*, *P. clausa* var. *immuginata*, *P. virginiana*, *P. palustris*, *P. ponderosa*, *P. ponderosa* var. *ponderosa*, *P. strobus*, *P. nigra*, *P. resinosa*, *Pseudotsuga menziesii*, *Quercus rubra*, *Fusarium moniliforme* var. *subglutinans*, *Pythium* spp., nematodes, seedling quality, artificial regeneration, nursery cultural practices, pesticides, pitch canker.

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## INTRODUCTION

THE CONCEPT OF FORMING ECTOMYCORRHIZAE on tree seedlings in nurseries with specific fungi ecologically adapted to the planting site to improve field performance of the seedlings was originally developed by Moser (1958) in Austria. This subject has been reviewed comprehensively by Bowen (1965), Mikola (1973), Trappe (1977), Marx (1980), and Molina and Trappe (1982). Using various modifications of Moser's techniques, Takacs (1967) in Argentina, Theodorou and Bowen (1970) in Australia, and Vozzo and Hacskeylo (1971) in the United States showed that field survival and growth of tree seedlings with specific ectomycorrhizae exceeded the performance of seedlings that lacked or had few native ectomycorrhizae at planting. Since 1966 research has been done by the USDA Forest Service in the southern United States to determine the significance of ectomycorrhizae formed by *Pisolithus tinctorius* (Pers.) Coker and Couch to survival and growth of tree seedlings on a variety of sites. This interest was prompted by the comprehensive report of Schramm (1966) on plant colonization of anthracite coal wastes in Pennsylvania, and observations by him and others (Lampky and Peterson 1963, Meyer 1968, Hile and Hennen 1969, Marx 1977a, Medve and others 1977) that

fruit bodies and ectomycorrhizae formed by *P. tinctorius* are found in great abundance on various tree species growing on coal and kaolin spoils, borrow pits, and other adverse sites. These sites are normally characterized by high soil temperatures during summer, extreme acidity, droughtiness, low fertility, or high levels of toxic metals. Usually, seedlings with *P. tinctorius* ectomycorrhizae were the most vigorous seedlings found on these sites and the yellow-gold ectomycorrhizae formed by this fungus were the first detected on roots of volunteer seedlings. Laboratory and growth room studies explained the persistence of *P. tinctorius* on these sites. In pure culture, the fungus was capable of growing at 40° to 42°C and grew most rapidly at 28° to 30°C (Marx and others 1970, Momoh and Gbadegesin 1980, Hung and Chien 1978). The thermal death point of *P. tinctorius* hyphae was 45°C (Lamb and Richards 1971). Other ectomycorrhizal fungi tested had much lower temperature tolerances and optima. Under aseptic conditions, loblolly pine (*Pinus taeda* L.) seedlings formed more ectomycorrhizae with *P. tinctorius* at 34°C than at lower temperatures (Marx and others 1970). Also, aseptically grown loblolly pine seedlings ectomycorrhizal with *P. tinctorius* survived and grew better at 40°C in laboratory tests than nonmycorrhizal seedlings or those ectomycorrhizal with *Thelephora terrestris* (Ehrh.) Fr. (Marx and Bryan 1971). These observations and results indicated that *P. tinctorius* could be a biological tool to improve survival and growth of pines on both poor quality forestation sites and reclamation sites. The fungus has several characteristics which make it suitable for practical application. It can easily be propagated in the laboratory on a variety of solid or liquid media. The yellow-gold or mustard color of its hyphae facilitates detection and quantitative assessment of ectomycorrhizae on seedling roots. It also produces abundant hyphal strands in pure culture and on seedling roots. Hyphal strands develop as extramatrical growth from roots into the surrounding soil, and may increase the nutrient (Bowen 1973) and water (Duddridge and others 1980) absorbing efficiency of ectomycorrhizal fungi. Fruit bodies of *P. tinctorius* are readily identified without microscopic examination because brown-yellow spores produced in compartments (peridioles) are a unique characteristic. This fungus has a proven host range of over 50 tree species and, under field conditions, has been associated with an additional 25 tree species; it has been reported from over 33 countries of the world and 38 of the United States (Marx 1977b). In addition to adverse sites, it is found in urban areas, orchards, many forest sites, and occasionally in tree nurseries (Grand 1976, Marx 1977b, Malloch and Kuja 1979). Since this fungus is ecologically adapted to adverse soil conditions which characterize most reclamation sites and many temporarily adverse reforestation sites (Schultz 1979), pine seedlings with *P. tinctorius* ectomycorrhizae should have a survival and growth advantage over seedlings with ectomycorrhizae formed by other fungi that occur naturally in nurseries.

One of the most common fungi occurring naturally in forest nurseries throughout the world is *Thelephora terrestris* (Weir 1921, Hacskeylo 1965, Mikola 1973). It is ecologically adapted to the excellent tilth, fertility, and moisture conditions of nursery soil but it often fails to adapt to the harsh soil conditions of outplanting sites. Because of adaptation to nursery soil environment, *T. terrestris* and other naturally occurring fungi are major competitors of introduced pure-culture inoculum of ectomycorrhizal fungi for seedling roots.

Marx (1980) discussed the early testing and development of vegetative inoculum of *P. tinctorius* for research. The procedure involved growing mycelium of the fungus for 3 or 4 months at room temperature in 1.5-liter jars containing a mixture of vermiculite and peat moss moistened with liquid medium. The substrate permeated by the fungus is removed from the jars, leached in tap water, and dried to a moisture content of 12 to 20 percent. Dried inoculum has been stored in small quantities, without losing significant viability, for 5 weeks at room tem-

perature and up to 9 weeks at 5°C. Mycelium which develops in the laminated structure of the vermiculite particles maintains viability and is protected from environmental stress and microbial saprophytic competition in the nursery soil. Mycelium of this fungus grown in other substrates, such as sand, perlite, peat moss, and cereal grains, has little or no protection from these factors.

Vermiculite-based inoculum of *P. tinctorius* has formed ectomycorrhizae in fumigated nursery soil on pine, oak, and pecan seedlings in microplots (Marx and Bryan 1975, Krugner 1976, Marx 1979a, b, c) and on pine seedlings in conventional bare-root nurseries in Georgia, Florida, and North Carolina (Marx and others 1976), Virginia (Marx and Artman 1978), Oklahoma (Marx and others 1978, 1979), Mississippi (Marx 1980), and Missouri (Dixon and others 1981b). In most of these nursery tests, seedlings with abundant *P. tinctorius* ectomycorrhizae grew larger than control seedlings with naturally occurring ectomycorrhizae. Fumigation of nursery soil prior to inoculation improves ectomycorrhizal development because it lowers populations of (1) soil microorganisms that can colonize introduced inocula (Bowen and Theodorou 1979, Marx 1980); (2) feeder root pathogens that damage roots and thus reduce ectomycorrhizal development (Ruehle 1973, Marx and others 1976); and (3) indigenous competing ectomycorrhizal fungi such as *T. terrestris* from previous tree crops (Marx and others 1976, 1978). Vermiculite-based inoculum broadcast at a rate of about 1 liter/m<sup>2</sup> of soil surface and mixed in soil has been as effective as higher rates in forming abundant *P. tinctorius* ectomycorrhizae in southern nurseries (Marx 1980).

Vermiculite-based inoculum also has been used successfully in forming *P. tinctorius* ectomycorrhizae on container-grown tree seedlings in various media and container types (Marx and Barnett 1974; Marx 1975; Ruehle and Marx 1977; Molina 1979, 1980; Dixon and others 1979, 1981b; Maronek and Hendrix 1980; Maronek and others 1981, 1982; Pawuk and others 1980; Ruehle and others 1981; Riffle and Tinus 1982; Grossnickle and Reid 1982).

The value of *P. tinctorius* ectomycorrhizae has been shown by the performance of seedlings under diverse field conditions. Significant, and often dramatic, enhancement of survival and growth of pine seedlings with abundant *P. tinctorius* ectomycorrhizae in comparison to seedlings with natural ectomycorrhizae produced in containers or bare-root nurseries have been reported from studies on acid coal spoils in Appalachia (Marx 1977a, Marx and Artman 1979, 1982, Berry 1982), kaolin spoils in Georgia (Marx 1977a, Otrosina 1977), severely eroded sites of the Copper Basin in Tennessee (Berry and Marx 1978), borrow pits in South Carolina (Ruehle 1980) and North Carolina (Goodwin 1982), and prairie soil (Baer and Otta 1981). Similar improvements in pine seedling performance were reported on routine reforestation sites in Florida and North Carolina (Marx and others 1977a), Oklahoma and Arkansas (Mexal 1980, Ruehle and others 1981), Georgia (Marx 1979a), Mississippi (Kais and others 1981), Louisiana (Vermillion 1982), and with seedlings of *Pinus caribaea* planted on afforestation sites in Nigeria (Momoh and Gbadegesin 1980), Congo (Delwaulle and others 1982), and Liberia (Marx, unpublished data). Recently, Dixon and others (1981a) reported that container-grown oak seedlings with *P. tinctorius* ectomycorrhizae survived and produced more shoot and root growth than noninoculated oak seedlings after 1 year on a reforestation site in Missouri. However, on poor soils amended with fertilizer or sewage sludge, pine seedlings with *T. terrestris* ectomycorrhizae have survived and grown as well as those with abundant *P. tinctorius* ectomycorrhizae (Marx and others 1977a, Berry and Marx 1980). Also, no effect of *P. tinctorius* ectomycorrhizae was observed with pine and spruce on a high elevation, molybdenum tailing pond (pH 8.0) in Colorado (Grossnickle and Reid 1982), with pines on a grass land (pH 8.4) in North Dakota (Riffle and Tinus 1982), with pine on a fertile abandoned field in Georgia (Powers and Rowan

1983), or with bare-root oak seedlings on a reforestation site in Missouri (Dixon and others 1981a).

To obtain maximum promotion of growth of seedlings of southern pines by *P. tinctorius* on reforestation sites, a threshold level of at least half of all ectomycorrhizae must be those of *P. tinctorius* at planting time (Marx and others 1977a, Kais and others 1981, Ruehle and others 1981, Ruehle and Brendemuehl 1981). Frequently, pine seedlings with less than half of all ectomycorrhizae formed by *P. tinctorius* grew at the same rate as seedlings with the same amount of *T. terrestris* ectomycorrhizae on routine reforestation sites.

Use of *P. tinctorius* on an operational basis for forest regeneration programs has been prevented by the lack of sufficient quantities of effective inoculum. In 1976, the Institute for Mycorrhizal Research and Development (IMRD) and State and Private Forestry, USDA Forest Service, and Abbott Laboratories, North Chicago, Illinois, began a cooperative program of research and nursery evaluation to develop commercial methods of producing vegetative inoculum of *P. tinctorius* and to test the effectiveness of different inoculum formulations under diverse nursery cultural regimes on different tree species throughout the United States. A comprehensive report was published on the testing of various formulations of the commercial inoculum for ectomycorrhizal development on container-grown tree seedlings of various species (Marx and others 1982). IMRD and Abbott inocula formed *P. tinctorius* ectomycorrhizae on 10 pine species, Douglas-fir, western hemlock, and bur oak in tests using different type containers and cultural practices at six locations in the United States and one in Canada. From the Canadian test, Navratil and others (1981) reported that the container-grown pine seedlings with *P. tinctorius* ectomycorrhizae formed by 1978 formulations of IMRD and Abbott inocula grew significantly more than initially larger, noninoculated seedlings after one growing season on a boreal forest site in Ontario.

This monograph reports a series of nursery studies carried out in conjunction with the container-grown seedling tests, which led to successful techniques for commercial production of pure-culture inoculum and for inoculating bare-root tree seedlings with *P. tinctorius*. Vegetative inoculum was produced by various procedures and types of commercial fermentation equipment at Abbott Laboratories. In each test, vegetative inoculum produced by research methods at the IMRD was used as a standard.

Various cultural practices routinely employed in tree nurseries can affect either ectomycorrhizal development or seedling size. Factors such as use of fumigants (Iyer and Wilde 1965, Iyer and Wojahn 1976, Sinclair 1974), pesticides (Iyer and others 1980; Smith and Ferry 1979; Cudlin and others 1980; Palmer and others 1980; Kelley 1980, 1982; Marx and Rowan 1981), fertilizers (Bowen 1973, Krugner 1976, Marx and others 1977b), rotation crops (Sinclair 1974, Iyer 1980), and seedling density (Harms and Langdon 1977) were monitored in the test nurseries. Attempts to correlate these cultural practices with success or failure of inoculations were made.

## 1977 TESTS

### MATERIALS AND METHODS

Unless otherwise stated, the following procedures, plot layouts, and experimental design were used throughout this investigation.

#### *Inoculum Production*

A single isolate of *P. tinctorius* (Pt), able to form abundant ectomycorrhizae on a variety of tree hosts under different conditions, was used throughout this in-

vestigation (Marx and others 1982). This was done to avoid confounding effects of fungal genotype on the ability of isolates to form ectomycorrhizae (Molina 1979; Marx 1979b, 1981). A fresh isolate was obtained annually by reisolating directly from ectomycorrhizae (Marx 1981) formed by IMRD inoculum on loblolly pine seedlings grown at the IMRD. Designation of this isolate was 155, 227, 246, and 250 for 1977, 1978, 1979, and 1980, respectively.

IMRD inoculum of Pt was produced using the following procedures. Pt was grown at 25°C for 3 wk in plates of modified Melin-Norkrans (MMN) agar medium (Marx 1969). Mycelium agar discs (8 mm dia) were used to initiate mass cultures for inoculum. The inoculum containers were 2-l jars containing a mixture of 1450 cc of grade 2 vermiculite, 50 cc of finely divided peat moss, and 750 cc of liquid MMN medium. MMN contains 0.05 g CaCl<sub>2</sub>, 0.025 g NaCl, 0.5 g KH<sub>2</sub>PO<sub>4</sub>, 0.25 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.15 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 1.2 ml of 1% FeCl<sub>3</sub>, 100 µg thiamine HCl, 3 g malt extract, and 10 g glucose in distilled H<sub>2</sub>O to equal a liter. Fifteen g of agar/l are added for agar formulation. After autoclaving, the pH of both the liquid and agar formulations is 5.5 to 5.7 (Marx 1969). The lid of each culture jar was modified with a 1-mm-thick, heat resistant, plastic disc through which a pyrex glass tube (6 × 2 cm) filled with cotton was inserted. The disc and tube joint was attached with a heat resistant, silicone rubber compound. The disc with tube was then attached to the metal threaded lid with the same adhesive. The containers were autoclaved 30 min and each inoculated with eight mycelium-agar discs of Pt. The lids were then wrapped with parafilm to reduce microbial contamination. After 15 to 20 wk at room temperature, the vermiculite particles were permeated with mycelium of Pt as determined by microscopic examination.

To prepare IMRD inoculum for infestation of soil, mycelium was removed from 4 to 6 jars and held with two layers of cheesecloth while being leached with approximately 8 liters of cool running tapwater. Excess water was removed by squeezing (Marx and Bryan 1975). The leached inoculum was placed 5 to 6 cm deep on wood framed wire screens (common window screen reinforced with a heavy gauge wire) and dried at 20° to 29°C and 35 to 45 percent relative humidity (Marx and Rowan 1981). During drying, the inoculum was turned by hand every 2 to 4 hr to minimize excessive drying of the surface particles. Drying was done in a small room (23 m<sup>3</sup> volume) constructed of clear polyethylene plastic containing a small air conditioner, two dehumidifiers, and a small heater. From 80 to 100 hr were needed to dry the IMRD inoculum properly. Bulk density (g/l) was determined by loosely filling a liter container and weighing. Moisture content (percent) was determined by drying 100 g of inoculum at 85°C for 48 hr. Difference in weights between the fresh and oven-dried inoculum was considered the moisture content.

All inocula reported herein were examined microscopically for relative amounts of hyphae of Pt inside and outside the vermiculite particles. This was done by placing several particles 3 to 5 mm diameter on a glass slide and saturating them with several drops of phloxine-lactophenol. After a few minutes, a cover slip was firmly placed on top of the particles to crush them. Fifteen to 20 fields of observations at 50× were made per slide for hyphae with clamp connections. Hyphae with clamp connections were assumed to be those of Pt. Ten to 15 slides were prepared per inoculum batch. Viability of hyphae was not determined with this technique.

The 1977 Abbott inoculum was produced in a vertical deep-tank, solid-substrate fermentor. The substrate contained vermiculite and MMN liquid medium with slightly more than recommended amounts of carbohydrates and organic and inorganic nitrogen. The substrate was placed in a fermentor, steamed (72° to 84°C), cooled, and inoculated with starter mycelium of Pt. Starter mycelium had been grown in a deep-tank, aerated submerged culture containing the same liquid medium. The culture was incubated in the fermentor, removed, and dried. In-

TABLE 1. Characteristics of vegetative inoculum of *Pisolithus tinctorius* produced by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories in 1977.

Inoculum source	Bulk density (g/l)	Moisture content (%)
IMRD		
Batch 1	342	42
Batch 2	376	59
Batch 3	362	48
Batch 4	352	43
Batch 5	355	43
Abbott		
Batch 1	288	21
Batch 2	205	15

oculum was not leached. Bulk density and moisture content were determined (Table 1). Abbott inoculum was shipped by air in drums from Chicago, IL, to the IMRD. Inoculum from both sources was packed in polyethylene bags at the IMRD to specific volumes according to treatment needs and stored at 5°C until shipment or use. Inoculum was transported to the nurseries in insulated chests containing artificial ice packs.

#### *Soil Management and Assay*

Nursery soil was fumigated with methyl bromide-chloropicrin formulations under clear polyethylene in the fall or spring preceding installation of each test. To determine effectiveness of fumigation, soil samples were collected from each test block (replicate) before and after fumigation, and shipped to the IMRD in insulated chests containing artificial ice packs. Upon arrival, soil samples were assayed for *Pythium* and *Phytophthora* spp. using modified Kerr's medium (Hendrix and Kuhlman 1965) and the procedure of Marx (1973), and for nematodes<sup>1</sup> using a centrifugal-flotation technique (Jenkins 1964). Soil samples were collected from 0 to 15 cm depth in several locations per block and combined into one composite sample per block. Soil samples were assayed for organisms within 7 days after collection. For chemical analyses, the post-fumigation soil samples were air dried at room temperature, screened to remove particles larger than 4 mm, and extracted with a double acid solution (0.05 N HCl + 0.025 N H<sub>2</sub>SO<sub>4</sub>). Phosphorus (P) was determined colorimetrically and cations by atomic absorption. Total N was determined by Kjeldahl, organic matter by wet oxidation chromic acid digestion, and pH by glass electrode in a soil paste.<sup>2</sup> A 3-year history of the study area was obtained from each nursery, including information on previous tree crops, cover crops, organic amendments, and fertilizers added before installation of each study.

#### *Plot Layout, Design, and Maintenance*

After soil fumigation and aeration, the soil was shaped into nursery beds. Five nursery beds (each a replicate block) varying in length from 150 to 350 m were

<sup>1</sup> Nematode extraction and identification by Dr. John L. Ruehle, USDA Forest Service, IMRD, Forestry Sciences Laboratory, Athens, GA 30602.

<sup>2</sup> Soil analyses by Dr. Carol G. Wells, USDA Forest Service, Forestry Sciences Laboratory, Research Triangle Park, NC 28807.

used; in most nurseries, each test bed was the second one from the irrigation riser so as to standardize irrigation rate. Starting about 14 m from the end of the nursery bed, five 1.2- × 1.2-m plots were arranged with 4-m spacing between plots. Treatments were randomly assigned to plots and the appropriate inoculum was broadcast evenly over the soil surface of the plot, mixed into the upper 10 cm of soil with hand tools, and the soil leveled. Control plots did not receive inoculum nor was the soil disturbed simulating inoculum incorporation unless otherwise stated. Inoculum medium without the fungus or killed IMRD inoculum does not affect pine seedling growth in nurseries (Marx 1980) and, therefore, was not added to soil of control plots except in the 1980 tests. Each nursery bed was seeded and mulched according to procedures used at the specific nursery. Hydro-mulch was used in many nurseries; it is a paper pulp suspended in water and blown onto the beds at specific rates after sowing seed. Seed and mulch were removed from approximately 30 cm of both ends of each plot. This seedling-free area on each end of the plots made the plots more visible later in the growing season and served as a visual reminder to nursery personnel that it was a study area. All plots received at least 2.5 cm of water each week either by irrigation or rainfall.

In the 1977 studies, five treatments were tested: three rates of Abbott inoculum (1.62, 1.08, and 0.54 l/m<sup>2</sup> of soil surface), one rate of IMRD inoculum (1.08 l/m<sup>2</sup>), and a control.

During the growing season, plots were observed for fruit bodies of ectomycorrhizal fungi. In most tests, those formed by *Pt* were removed, measured, noted as stalked or sessile, and recorded. *Pt* fruit bodies were recorded to correlate their incidence with degree of *Pt* ectomycorrhizal development and they were removed from plots to minimize contamination of other plots. Fruit bodies of other fungi were recorded but not removed.

#### *Seedling Samples and Assessments*

Midstudy root assessments were made to obtain an early indication of inoculum effectiveness and to ascertain the degree of competition by naturally occurring ectomycorrhizal fungi. Five representative seedlings were removed at random from each plot in late July, August, or early September for seedlings lifted as 1-0 stock, or at the end of the first or second growing season for those lifted as 2-0 or 3-0 stock, respectively. The seedlings were visually assessed for ectomycorrhizal development at the nursery or packed and shipped to the IMRD in insulated chests with artificial ice packs for examination.

At the termination of each study, all seedling plots were undercut with a root-pruning bar at a depth of 15 to 25 cm. Seedlings were lifted by hand from the soil and immediately placed in containers with adequate moisture to keep them moist. All seedlings lifted from each plot were counted and graded into culls and plantables according to the height and root-collar diameter standards of each nursery. Ten plantable seedlings per plot were chosen at random and were assessed within 24 hr at the nursery or were wrapped in wet paper towels, placed in treatment-labeled plastic bags, packed in insulated chests with artificial ice packs, and shipped to the IMRD for assessment within 8 days. Height, root-collar diameter, and top and root fresh weights were measured and the degree of ectomycorrhizal development on each seedling was visually assessed without magnification (Marx and Bryan 1975).

Data on ectomycorrhizae were integrated into a *Pt* index using the formula  $a \times (b/c)$  where  $a$  = percent of assessed seedlings with *Pt* ectomycorrhizae,  $b$  = average percent of feeder roots with *Pt* ectomycorrhizae (including 0 percent for seedlings without *Pt*), and  $c$  = average percent of feeder roots with ectomycorrhizae formed

by Pt and other fungi (total ectomycorrhizal development). An index of 100 means that all the ectomycorrhizae formed on all the assessed seedlings were formed by Pt. An index of 50 indicates that the majority of seedlings have more than half of the ectomycorrhizal root system dominated by Pt. This index, which integrates all measurements on Pt ectomycorrhizal development into a single value, provides a measure of the viability and effectiveness of the inoculum (Marx 1981, Marx and others 1982). A Pt index of 50 was considered to be the lowest acceptable value for effective inoculum since field results on reforestation sites showed that southern pine seedlings only obtained significant benefit from Pt when at least half of all ectomycorrhizae on their roots at planting were formed by that fungus (Marx and others 1977a, Ruehle and others 1981, Kais and others 1981).

All data were processed by analysis of variance, and significant differences among means were identified with Duncan's New Multiple Range Test at  $P = 0.05$ .

#### NURSERY INFORMATION AND RESULTS

In 1977 and in other years, one or more nursery tests were installed but not carried to completion because of problems such as heavy rains washing seed from test plots, uneven application of organic amendments (sawdust, wood chips) to plots before inoculation causing nitrogen deficiencies and poor seedling growth, root damage from improper root pruning or lifting seedlings from frozen or waterlogged soil, inadvertent lifting of test seedlings by nurserymen for use as operational seedlings, in-transit loss of chests containing test seedlings, and others. These tests are not included in this paper.

Cropping history and the cultural practices involved in the 1977 nursery tests before study installation are given in Appendix I, chemical and physical characteristics of soil at study installation are given in Appendix II, and cultural practices involved after study installation are in Appendix III; other details are as follows:

*W. A. Ashe Nursery, Brooklyn, Mississippi.*—During soil fumigation in this USDA Forest Service nursery, day air temperature (sunny) was 27°C and soil moisture was approximately 50 percent of field capacity. Plastic covering was removed after 3 days. IMRD inoculum batch 3 was stored for 13 days and Abbott inoculum batch 1 was stored for 22 days before use. Inoculum was applied and nontreated seeds of shortleaf pine (*Pinus echinata* Mill.) were planted on April 27, 1977. Pulverized pine cones were spread 1 cm deep over soil as a mulch immediately after seeding. Midstudy root assessments were done in late July, and the study was terminated in February 1978. Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 5 mm. Successful inoculations of shortleaf pine and longleaf pine (*P. palustris* Mill.) with IMRD vegetative inoculum of Pt were reported earlier in this nursery (Marx 1980).

*Results:* Soil fumigation eliminated all organisms assayed (Table 2). Shortly after installation of the study, heavy rains washed numerous seeds from test plots causing erratic seedbed density (37 to 305 seedlings/m<sup>2</sup>) and a high number of cull seedlings (Table 3). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 55 and 0 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae, resembling those formed by *Thelephora terrestris*, were found on 9 to 17 percent of short roots on control seedlings; a few *T. terrestris* (Tt) fruit bodies were observed in all plots at that time. All Tt fruit bodies observed in this and other nurseries reported herein were quite similar in gross morphological characteristics.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 76 and included significantly fewer culls than controls or the high rate

TABLE 2. Number of Pythiaceae fungal propagules (pg) and plant parasitic nematodes isolated from pre- and post-fumigated soil samples in nursery tests in 1977.

Nursery	Pythiaceae fungi <sup>1</sup> pg/g		Nematodes/475 cc soil	
	Pre-fum	Post-fum	Pre-fum	Post-fum
Ashe, MS	2	0	23 <sup>2,3</sup>	0
Andrews, FL	9	0	392 <sup>3,4</sup>	24 <sup>3</sup>
Beauregard, LA	36	<1	3 <sup>3,4</sup>	0
Weyerhaeuser, OK (old area)	0	0	0	0
Weyerhaeuser, OK (new area)	23	0	2 <sup>3</sup>	0
Weyerhaeuser, AR	7	0	0	0
Buckeye, FL	32	0	2 <sup>2,3</sup>	<1 <sup>2</sup>
Great Southern, GA	15	0	52 <sup>3,5,6,7</sup>	0
Westvaco, SC	10	0	803 <sup>2,3,6</sup>	0
Kimberly-Clark, AL	53	0	13 <sup>2,4</sup>	0
Placerville, CA	142	29	0	0
Bessey, NE	4	0	5 <sup>2,4</sup>	0
New Kent, VA	7	0	1 <sup>3,4</sup>	0
Pinson, TN	102	0	0	0
Griffith, NC (Virginia pine)	101	12	2 <sup>2,4</sup>	0
Griffith, NC (Longleaf pine)	108	53	12 <sup>3,6</sup>	0
Kentucky Dam, KY	43	0	11 <sup>6</sup>	0
IMRD, GA (proper fumigation)	19	0	217 <sup>2,3,4</sup>	0
IMRD, GA (improper fumigation)	19	8	217 <sup>2,3,4</sup>	64 <sup>3,4</sup>

<sup>1</sup> Mainly *Pythium irregulare*.

<sup>2</sup> Stunt nematode (*Tylenchorhynchus*).

<sup>3</sup> Ring nematode (*Criconeimoides*).

<sup>4</sup> Saprophytic nematodes.

<sup>5</sup> Lesion nematode (*Pratylenchus*).

<sup>6</sup> Dagger nematode (*Xiphinema*).

<sup>7</sup> Root knot nematode (*Meloidogyne*).

of Abbott inoculum (Table 3). Abbott inoculum was ineffective in forming Pt ectomycorrhizae at all rates. Seedling growth was not significantly affected by treatment. Tt and Pt ectomycorrhizae occurred naturally on seedlings and both fungi formed fruit bodies in test plots, regardless of treatment. More Pt fruit bodies occurred in plots of IMRD inoculum. Tt formed fruit bodies on 21 percent of sampled seedlings. Even though soil fumigation appeared effective and captan was applied during the growing season, severe necrosis of feeder roots was observed on many seedlings, especially on the lower one-third of seedling roots. Apparently the fumigant and captan were only effective in the upper volume of soil.

*Andrews Nursery, Chiefland, Florida.*—During soil fumigation in this state nursery, soil temperature (sunny) was 24°C and soil moisture was less than 30 percent of field capacity. Plastic covering was removed after 2 days. IMRD inoculum batch 1 was stored for 2 days and Abbott inoculum batch 1 was stored for 8 days before use. Inoculum was applied and thiram-treated seeds of sand pine (*P. clausa* Chapm. ex Engelm.) var. *immuginata*) were planted on April 13, 1977. Seeds were covered with 784 kg/ha of hydromulch. Midstudy root assessments were made in late July, and the study was terminated in November. Seedlings were considered culls if shorter than 18 cm tall and with root-collar diameters less than 2 mm. Successful inoculations of sand pine, loblolly pine, and slash pine (*P. elliottii* Engelm. var.

TABLE 3. Growth and ectomycorrhizal development of pine seedlings from various nurseries with vegetative inoculum of *Pisolithus tinctorius* (Pt) produced in 1977 by the Institute for Mycorrhizal Research and Development (IMRD) and batches 1 and 2 produced by Abbott Laboratories.<sup>1</sup>

Location, pine species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ectomycor- rhizal with—		Per- cent seed- lings with Pt	Pt index <sup>2</sup>	Per- cent cull seed- lings
			Top	Root	Total	Pt	All fungi			
Ashe, MS: shortleaf										
IMRD 1.08	20.5a <sup>3</sup>	6.2a	13.1a	13.4a	26.7a	34a	43a	100a	76a	58b
Abbott #1, 1.62	19.9a	6.0a	12.2a	11.0a	23.2a	1b	35b	8c	<1b	50b
Abbott #1, 1.08	18.6a	5.3a	8.9a	8.7a	17.7a	5b	36b	30b	4b	56b
Abbott #1, 0.54	19.9a	5.9a	11.4a	10.6a	22.0a	2b	34b	8c	<1b	65a
Control	19.3a	5.8a	11.6a	9.7a	21.3a	3b	35b	24bc	2b	70a
Andrews, FL: sand										
IMRD 1.08	21.6a <sup>3</sup>	2.8a	7.9a	1.6a	9.5a	30a	35a	100a	87a	14b
Abbott #1, 1.62	21.4a	2.5a	6.6ab	1.4a	8.0ab	1b	14c	12b	<1b	31a
Abbott #1, 1.08	21.3a	2.6a	6.7ab	1.4a	8.1ab	1b	16c	8b	<1b	27a
Abbott #1, 0.54	20.2a	2.6a	6.6ab	1.3a	7.9ab	1b	15c	6b	<1b	29a
Control	20.6a	2.5a	5.6b	1.1a	6.7b	0	24b	0	0	32a
Beauregard, LA: longleaf										
IMRD 1.08	—	8.1a	15.1b	5.4b	20.6ab	26a	31a	100a	81a	19a
Abbott #1, 1.62	—	7.9b	12.7b	5.2b	17.9b	1b	22b	18b	<1b	17a
Abbott #1, 1.08	—	7.9b	14.4a	5.4b	19.8b	2b	19b	26b	3b	20a
Abbott #1, 0.54	—	7.8b	11.8b	4.7b	16.5b	1b	18b	10b	<1b	27a
Control	—	8.7a	18.3a	7.0a	25.3a	0	22b	0	0	11a
Weyerhaeuser, OK (old area): loblolly										
IMRD 1.08	20.8a <sup>3</sup>	4.0a	9.2a	2.4a	11.6a	7a	32a	76a	18a	24b
Abottt #1, 1.62	20.4a	3.9a	9.1a	2.6a	11.7a	1b	34a	6b	<1b	35ab
Abbott #1, 1.08	22.7a	4.2a	10.2a	2.8a	13.0a	1b	37a	5b	<1b	48a
Abbott #1, 0.54	20.7a	3.8a	8.7a	2.5a	11.2a	1b	40a	18b	<1b	42a
Control	20.3a	3.9a	9.0a	2.4a	11.4a	0	37a	0	0	42a
Weyerhaeuser, OK: (new area): loblolly										
IMRD 1.08	28.5a <sup>3</sup>	4.6a	11.8a	3.3a	15.1a	15a	37a	88a	37a	22a
Abbott #1, 1.62	28.5a	4.5a	10.7a	2.9a	13.6a	1b	36a	14b	<1b	24a
Abbott #1, 1.08	27.3a	4.5a	10.7a	2.9a	13.6a	1b	35a	12b	<1b	23a
Abboitt #1, 0.54	29.8a	4.8a	11.5a	3.4a	14.9ab	1b	36a	10b	<1b	27a
Control	28.2a	4.4a	10.6a	3.4a	14.0b	0	43a	0	0	20a
Weyerhaeuser, AR: loblolly										
IMRD 1.08	28.3a <sup>3</sup>	4.7a	12.4a	2.9a	15.3a	11a	27a	84a	36a	16b
Abbott #1, 1.62	22.3b	4.0a	8.8b	2.4a	11.2ab	2b	35a	20b	1b	25ab
Abbott #1, 1.08	23.3b	4.1a	9.4b	2.3a	11.7ab	3b	32a	18b	2b	28a
Abbott #1, 0.54	25.8b	4.6a	12.5a	3.0a	15.5a	1b	32a	8b	<1b	32a
Control	23.2b	4.4a	10.5ab	2.7a	13.2ab	0	33a	0	0	30a

TABLE 3. Continued.

Location, pine species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ectomycor- rhizal with—		Per- cent seed- lings with Pt	Pt index <sup>2</sup>	Per- cent cull seed- lings
			Top	Root	Total	Pt	All fungi			
Buckeye, FL: slash										
IMRD 1.08	20.6a <sup>3</sup>	3.5a	7.2a	1.9a	9.1a	15a	28a	98a	53a	5b
Abbott #1, 1.62	19.2a	3.1ab	5.7b	1.3b	7.0b	1b	29a	2b	<1b	14a
Abbott #1, 1.08	19.5a	3.2ab	5.9b	1.3b	7.2b	1b	36a	4b	<1b	16a
Abbott #1, 0.54	19.7a	3.1ab	5.8b	1.2b	7.0b	1b	33a	2b	<1b	13a
Control	19.1a	3.0b	5.4b	1.2b	6.6b	0	28a	0	0	12a
Great Southern, GA: loblolly										
IMRD 1.08	29.4a <sup>3</sup>	4.5a	15.4a	2.8a	18.2a	53a	59a	100a	91a	7b
Abbott #1, 1.62	28.1a	4.0b	13.9b	2.3b	16.2b	31b	44b	90a	62b	12a
Abbott #1, 1.08	28.3a	3.8b	12.3b	1.9b	14.2bc	17c	39b	78b	31c	10ab
Abbott #1, 0.54	27.8a	3.9b	11.8b	2.0b	13.8c	9c	35b	64b	16c	10ab
Control	27.7a	3.8b	11.3b	1.8b	13.1c	1d	29c	12c	<1d	12a
Westvaco, SC: loblolly										
IMRD 1.08	29.3a <sup>3</sup>	5.2a	14.8a	3.7a	18.5a	37a	49a	100a	77a	7a
Abbott #1, 1.62	27.9ab	5.6a	14.9a	4.2a	19.1a	17b	44a	80b	31b	7a
Abbott #1, 1.08	25.9ab	5.5a	14.9a	4.4a	19.3a	10b	47a	68b	14c	6a
Abbott #1, 0.54	27.5ab	5.5a	14.2a	4.1a	18.3a	14b	49a	56b	15c	9a
Control	23.0b	5.3a	10.4b	2.9b	13.3b	1c	47a	6c	<1d	6a
Kimberly-Clark, AL: loblolly										
IMRD 1.08	33.8a	5.3a	16.8a	5.4a	22.2a	23a	43a	96a	52a	10a
Abbott #1, 1.62	34.1a	5.2a	15.2a	5.0a	20.2a	7b	48a	52b	8b	9a
Abbott #1, 1.08	33.8a	5.5a	14.9a	5.5a	20.4a	5b	47a	40b	4b	9a
Abbott #1, 0.54	33.9a	5.4a	14.3a	5.6a	19.9a	6b	46a	22c	3b	10a
Control	35.7a	5.4a	16.4a	5.0a	21.4a	0	43a	0	0	10a
Placerville, CA: ponderosa										
IMRD 1.08	10.2a	3.3a	3.2a	3.5a	6.7a	21a	27a	86a	69a	17c
Abbott #2, 1.62	10.3a	3.3a	3.1a	3.1a	6.2a	1b	19b	8b	1b	23b
Abbott #2, 1.08	8.7a	3.1a	2.5a	3.2a	5.7a	0	20b	0	0	25b
Abbott #2, 0.54	10.0a	3.2a	3.0a	3.5a	6.5a	0	18b	0	0	22b
Control	10.0a	3.4a	3.4a	3.5a	6.9a	0	18b	0	0	30a
Bessey, NE: ponderosa (2–0)										
IMRD 1.08	24.2a	7.5a	26.5a	11.4a	37.9a	25a	30a	100a	86a	38b
Abbott #2, 1.62	23.6ab	7.0a	23.6a	9.7b	33.3b	5b	17b	62b	19b	41ab
Abbott #2, 1.08	23.8ab	6.9a	24.8ab	9.7b	34.5b	3b	18b	40c	7c	41ab
Abbott #2, 0.54	23.6ab	7.4a	26.2a	10.1ab	36.3ab	3b	20b	46c	7c	41ab
Control	22.9b	7.3a	23.3b	9.5b	32.8b	0	10c	0	0	42a
New Kent, VA: loblolly										
IMRD 1.08	18.6a <sup>3</sup>	4.2a	6.7a	3.1a	9.8a	20a	38a	100a	55a	6a

TABLE 3. Continued.

Location, pine species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ectomycor- rhizal with—		Per- cent seed- lings with Pt	Pt index <sup>2</sup>	Per- cent cull seed- lings
			Top	Root	Total	Pt	All fungi			
Abbott #2, 1.62	18.0a	4.0a	6.1ab	3.0ab	9.1ab	2b	42a	12b	<1b	6a
Abbott #2, 1.08	18.5a	4.0a	5.9ab	3.0ab	8.9ab	0	42a	0	0	6a
Abbott #2, 0.54	18.5a	3.8a	5.8ab	2.8ab	8.6ab	0	45a	0	0	6a
Control	18.2a	3.9a	5.3b	2.5b	7.8b	0	45a	0	0	7a
Pinson, TN:										
Virginia										
IMRD 1.08	18.3a <sup>3</sup>	3.8a	11.5a	4.1a	15.6a	44a	51a	100a	88a	16c
Abbott #2, 1.62	15.8b	3.4b	8.9b	3.8ab	12.7b	4b	40b	40b	5b	70a
Abbott #2, 1.08	16.9ab	3.3b	8.0b	3.2ab	11.2b	1b	34b	10c	<1b	28b
Abbott #2, 0.54	16.6ab	3.3b	8.0b	2.9b	10.9b	1b	37b	8c	<1b	39b
Control	15.5b	3.2b	7.7b	3.3ab	11.0b	0	37b	0	0	25b
Griffith, NC:										
Virginia										
IMRD 1.08	24.6a	3.9a	12.5a	5.1a	17.6a	46a	52a	100a	89a	7b
Abbott #2, 1.62	18.4b	3.3b	9.1b	3.5b	12.6b	1b	31b	12b	<1b	17a
Abbott #2, 1.08	21.4ab	3.5b	10.4b	3.9b	14.3ab	2b	34b	10b	<1b	15a
Abbott #2, 0.54	22.2ab	3.3b	9.6b	3.5b	13.1b	1b	32b	2b	<1b	17a
Control	20.6b	3.4b	10.8b	3.7b	14.5ab	0	33b	0	0	16a
Griffith, NC:										
longleaf										
IMRD 1.08	—	10.3a	22.1a	12.9a	23.9a	8a	13a	96a	54a	8a
Abbott #2, 1.62	—	10.0a	19.2a	10.8a	30.0a	0	10a	0	0	8a
Abbott #2, 1.08	—	10.2a	20.0a	11.3a	31.3a	1b	11a	6b	<1b	7a
Abbott #2, 0.54	—	9.4a	18.5a	10.5a	29.0a	0	8a	0	0	7a
Control	—	9.7a	20.4a	11.7a	32.1a	0	9a	0	0	11a
Kentucky Dam, KY:										
shortleaf										
IMRD 1.08	27.5a <sup>3</sup>	6.3a	16.9a	7.0a	23.9a	26a	41a	96a	58a	24c
Abbott #2, 1.62	26.9a	6.3a	18.0a	6.1ab	24.1a	6b	35a	48b	9b	33b
Abbott #2, 1.08	25.2a	5.7b	15.4a	5.5b	20.9b	3b	33a	34b	3b	36b
Abbott #2, 0.54	27.1a	5.7b	15.2a	4.8b	20.0b	6b	34a	42b	7b	40b
Control	25.8a	6.0ab	16.0a	6.3ab	22.3a	0	41a	0	0	56a

<sup>1</sup> Means sharing a common letter in the same nursery but between inoculum treatments are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a(b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> Seedling tops mowed to approximately 20 cm at least once during growing season.

*elliottii*) with IMRD vegetative inoculum of Pt were reported earlier in this nursery (Marx and others 1976).

**Results:** Soil fumigation eliminated the low numbers of Pythiaceae fungi but not all nematodes (Table 2). Midstudy assessments of ectomycorrhizae on the

seedlings revealed Pt indices of 76 and 0 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae resembling Tt were found on 15 to 23 percent of short roots on control seedlings at that time.

At termination of the study, IMRD inoculum had resulted in a Pt index of 87, significantly increased seedling growth, and significantly reduced the number of seedling culls in comparison to control seedlings (Table 3). Abbott inoculum not only was ineffective in forming Pt ectomycorrhizae, it also significantly reduced the incidence of naturally occurring ectomycorrhizae. Fruit bodies of Tt occurred erratically on seedling stems, and it appeared to be the major naturally occurring ectomycorrhizal fungus on roots. Incidence of fruit bodies of Pt was not recorded. Seedling density averaged 254 seedlings/m<sup>2</sup> and was not affected by treatment.

*Beauregard Nursery, DeRidder, Louisiana.*—During soil fumigation in this state nursery, soil temperature at 15 cm depth (overcast) was 18°C and soil moisture was 10 percent by weight. Plastic covering was removed after 4 days. IMRD inoculum batch 3 was stored for 5 days and Abbott inoculum batch 1 was stored for 14 days before use. Inoculum was applied and thiram-treated seeds of longleaf pine were planted on April 19, 1977. Seeds were covered with a 2-cm layer of fumigated pine straw mulch. Midstudy root assessments were made in early August, and the study was terminated in early December. Seedlings were considered culls with root-collar diameters less than 7 mm.

*Results:* Soil fumigation eliminated all but a few Pythiaceae fungi (Table 2). One day after installation of the study, more than 50 cm of rain fell on the nursery during a 5-day period. This rain not only washed seed from the nursery beds but also actually destroyed certain plots. Control plots which did not have soil disturbed at study installation, in contrast to the inoculated plots, were not damaged as severely. Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 57 and <1 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae resembling Tt were found on 9 to 16 percent of short roots on control seedlings at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 81; Abbott inoculum was ineffective (Table 3). Bed damage by rain caused erratic seedbed densities (116 to 236 seedlings/m<sup>2</sup>), resulting in highly variable seedling growth. Control seedlings were actually larger than seedlings in the Abbott inoculum treatments. Fruit bodies of Tt occurred on stems of nearly 20 percent of sampled seedlings regardless of treatment. Fruit bodies of Pt were not recorded.

*Weyerhaeuser Company Nursery, Fort Towson, Oklahoma.*—Tests were installed in an old and new area in this forest industry nursery. During soil fumigation in both areas, soil temperature at 15 cm depth (sunny) was 14°C and soil moisture was approximately 60 percent of field capacity. Plastic covering was removed after 3 days. IMRD inoculum batch 1 was stored for 18 days and Abbott inoculum batch 1 was stored for 21 days before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 28, 1977, in both studies. Seeds were covered with 1,680 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in late December. Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 3 mm. Successful inoculation of loblolly pine with various inocula of Pt was reported in this nursery earlier (Marx and others 1978, 1979).

*Results:* Soil fumigation eliminated all nematodes in the new nursery section, and there were no Pythiaceae fungi or nematodes in the old nursery section even before soil fumigation (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 44 and 0 in the new nursery section and 15 and <1 in the old nursery section for IMRD and Abbott inoculum treatments,

respectively. Naturally occurring ectomycorrhizae resembling those formed by Tt were found on 9 to 16 percent and 26 to 34 percent of short roots on control seedlings in the old and new nursery sections, respectively.

At termination of the study in both the old and new nursery sections, the Pt index of seedlings was <50 for all inoculum treatments (Table 3). Nearly twice as much Pt ectomycorrhizae occurred on seedlings from IMRD inoculum in the new nursery section as in the old section, but seedling growth was not affected. However, IMRD inoculum in the old section significantly reduced seedling culls in comparison to control seedlings. Seedling density in the new nursery section averaged 370 seedlings/m<sup>2</sup> and the old section averaged 451 seedlings/m<sup>2</sup>; density was not affected by inoculum treatment. Tt formed abundant ectomycorrhizae on all seedlings and formed fruit bodies on 15 percent of sampled seedlings in the new nursery section and on 24 percent of sampled seedlings in the old nursery section. Incidence of fruit bodies of Pt was not recorded.

*Weyerhaeuser Company Nursery, Magnolia, Arkansas.*—During soil fumigation in this forest industry nursery, day air temperature (overcast) averaged 21°C and soil moisture was about 50 percent of field capacity. Plastic covering was removed after 2 wks. IMRD inoculum batch 1 was stored for 17 days and Abbott inoculum batch 1 was stored for 20 days before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 25, 1977. Seeds were covered with 1,500 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in February 1978. Seedlings were considered culls if shorter than 18 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 53 and 0 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae resembling Tt were observed on 9 to 16 percent of short roots on control seedlings at that time.

At termination of the study, seedlings that received either source of inoculum did not have a Pt index >50 (Table 3). Pt ectomycorrhizae from IMRD inoculum, however, significantly increased seedling size and significantly reduced culls by nearly half in comparison to control seedlings. Seedling density averaged 418 seedlings/m<sup>2</sup> and was not affected by treatment. Tt formed abundant ectomycorrhizae on all seedlings and formed fruit bodies on 29 percent of the sampled seedlings. Incidence of Pt fruit bodies was not recorded.

*Buckeye Cellulose Corporation Nursery, Perry, Florida.*—During soil fumigation in this forest industry nursery, day air temperature (sunny) averaged 22°C, and soil moisture was 7.7 percent by weight. Plastic covering was removed after 3 days. IMRD inoculum batch 2 was stored for 2 days and Abbott inoculum batch 1 was stored for 8 days before use. Inoculum was applied and nontreated seeds of slash pine were planted on April 13, 1977. Seeds were covered with 1,120 kg/ha of hydromulch. Midstudy root assessments were made in late July, and the study was terminated in November. Seedlings were considered culls if shorter than 13 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all but a few nematodes (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 18 and 0 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae, predominantly a white, complex coralloid type, was found on 23 to 37 percent of short roots on control seedlings. A few *Rhizopogon nigrescens* N. sp. fruit bodies were found in plots at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 53; Abbott inoculum was ineffective (Table 3). Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth and significantly reduced

culls by over half in comparison to control seedlings. Seedling density averaged 417 seedlings/m<sup>2</sup> and was not affected by treatment. Sixteen Pt fruit bodies were found in plots of IMRD inoculum; size ranged from 2 to 14 cm in diameter and three had stalks. Tt formed about half of the naturally occurring ectomycorrhizae, and it produced fruit bodies on 11 percent of all sampled seedlings. An average of eight fruit bodies of *R. nigrescens* occurred in each test plot. About half of the naturally occurring ectomycorrhizae appeared to be formed by *R. nigrescens*.

*Great Southern Paper Company Nursery, Cedar Springs, Georgia.*—During soil fumigation in this new forest industry nursery, day air temperature (sunny) averaged 24°C and soil moisture was approximately 60 percent of field capacity. Plastic covering was removed after 3 days. IMRD inoculum batch 2 was stored for 1 day and Abbott inoculum batch 1 was stored for 7 days before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 12, 1977. Seeds were covered with 1,560 kg/ha of hydromulch. Midstudy root assessments were made in late July, and the study was terminated in October. Seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Ft indices of 92, 26, 36, and 1 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae resembling those formed by Tt were found on 5 to 11 percent of short roots on control seedlings at that time.

At termination of the study, seedlings that received IMRD inoculum and Abbott inoculum at the highest rate had Pt indices of 91 and 62, respectively (Table 3). Seedlings in these treatments were also significantly larger than control seedlings. Only the IMRD inoculum, however, significantly reduced seedling culls in comparison to control seedlings. Seedling density averaged 344 seedlings/m<sup>2</sup> and was not affected by treatment. Totals of 5, 9, 2, and 3 fruit bodies of Pt were produced in IMRD and the three Abbott treatments, respectively. Fruit bodies ranged in size from 1 to 15 cm in diameter and all were stalked. Only a few Tt fruit bodies were observed on sampled seedlings. A few Pt ectomycorrhizae were observed on control seedlings.

*Westvaco Corporation Nursery, Summerville, South Carolina.*—During soil fumigation in this forest industry nursery, day air temperature (sunny to overcast) averaged 21°C and soil moisture was 65 percent of field capacity. Plastic covering was removed after 4 days. IMRD inoculum batch 2 was stored for 1 day and Abbott inoculum batch 1 was stored for 7 days before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 12, 1977. Seeds were covered with 1,680 kg/ha of hydromulch. Midstudy root assessments were made in late July, and the study was terminated in early November. Seedlings were considered culls if shorter than 13 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Ft indices of 82, 2, 3, and 2 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae resembling those formed by Tt were found on 11 to 19 percent of short roots on control seedlings at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 77; Abbott inoculum consistently formed ectomycorrhizae, but not in sufficient amounts to satisfy the minimum Pt index requirement (Table 3). The Pt ectomycorrhizae formed in all inoculum treatments significantly increased seedling growth but did not affect cull percentage in comparison to control seedlings. Seedling density averaged 280 seedlings/m<sup>2</sup> and was not affected by treat-

ment. Pt formed 15 stalked fruit bodies (2 to 6 cm in diameter) in IMRD inoculum plots but none in the Abbott inoculum plots. Tt fruit bodies occurred on 13 percent of test seedlings.

*Kimberly-Clark Corporation Nursery, Coosa Pines, Alabama.*—During soil fumigation in this forest industry nursery, day air temperature (sunny) averaged 27°C and soil moisture was approximately 50 percent of field capacity. Plastic covering was removed after 2 days. IMRD inoculum batch 1 was stored for 13 days and Abbott inoculum batch 1 was stored for 22 days before use. Inoculum was applied and seeds of loblolly pine were planted on April 27, 1977. Seeds were treated with anthraquinone (bird repellent), captan, and latex sticker. Seeds were covered with a 1-cm layer of nonfumigated pine bark as a mulch. Midstudy root assessments were made in late July, and the study was terminated in February 1978. Seedlings were considered culls if shorter than 20 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 75, 7, 3, and 3 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae were observed on 26 to 37 percent of short roots on control seedlings; a few Tt fruit bodies were observed at that time.

Seedlings were not undercut with a root-pruning bar before lifting and seedling roots were stripped of many ectomycorrhizae during hand lifting. Seedlings that received IMRD inoculum had a Pt index of 52; Abbott inoculum was ineffective (Table 3). No inoculum treatment significantly affected either seedling size or number of culls in comparison to control seedlings. Seedling density averaged 303 seedlings/m<sup>2</sup> and was not affected by treatment. Tt fruit bodies occurred erratically in all plots as did naturally occurring Pt in some control plots.

*Placerville Nursery, Camino, California.*—During soil fumigation in this USDA Forest Service nursery, soil temperatures at 15 cm depth were 8° to 19°C (overcast) and soil moisture was approximately 80 percent of field capacity. Plastic covering was removed after 3 days. IMRD inoculum batch 5 was stored for 4 days and Abbott inoculum batch 2 was stored for 13 days before use. Inoculum was applied and nontreated seeds of ponderosa pine (*P. ponderosa* Dougl. ex Laws.) were planted on May 2, 1977. Seeds were not covered with mulch. During the growing season various pesticides and fertilizers were applied to all plots, however, records of use were not available. Midstudy root assessments were made in late July, and the study was terminated in February 1978. Seedlings were considered culls if shorter than 5 cm, twisted, or broken.

*Results:* Soil fumigation did not eliminate all Pythiaceae fungi (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 10 and 0 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae were found on only 4 to 7 percent of short roots on control seedlings at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 69; Abbott inoculum was ineffective (Table 3). Seedling growth was not affected by any treatment, but both IMRD and Abbott inocula significantly reduced seedling culls in comparison to control seedlings. IMRD inoculum reduced culls by nearly half. Seedling density averaged 401 seedlings/m<sup>2</sup> and was not affected by treatment. The incidence of naturally occurring ectomycorrhizae was low (18 to 20 percent); fruit bodies of ectomycorrhizal fungi were not observed.

*Bessey Tree Nursery, Halsey, Nebraska.*—During soil fumigation in this USDA Forest Service nursery, day air temperature averaged 21°C and soil moisture was approximately 40 percent of field capacity. Plastic covering was removed after 8

Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 2). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 77, 6, 14, and 4 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt were found on 9 to 16 percent of short roots on control seedlings at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 88; Abbott inoculum was ineffective (Table 3). Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth and significantly reduced culls in comparison to control seedlings. The highest rate of Abbott inoculum increased the number of cull seedlings. Seedling density averaged 150 seedlings/m<sup>2</sup> and was not affected by treatment. Fruit bodies were not recorded during the growing season, but three Pt fruit bodies were observed at lifting time in plots of IMRD inoculum. Numerous Tt fruit bodies were observed in all plots at lifting time and this fungus apparently formed most of the naturally occurring ectomycorrhizae.

*Griffith Experimental Nursery, Clayton, North Carolina.*—Two tests, each with a different pine species, were installed in this state nursery. During soil fumigation, day air temperature averaged 34°C and soil moisture was approximately 30 percent of field capacity. Plastic covering was removed after 8 days. IMRD inoculum batch 4 was stored for 9 days and Abbott inoculum batch 2 was stored for 10 days before use. Inoculum was applied in both studies on April 29, 1977. Seeds of Virginia and longleaf pine were treated with thiram and latex sticker. Seeds were covered with a 1.5-cm layer of coarse ground wood as a mulch. Midstudy root assessments were made in late July and both studies were terminated in December. Virginia pine seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 3 mm. Longleaf pine seedlings with root-collar diameters less than 8 mm were considered culls.

*Results:* In the Virginia pine plots, soil fumigation eliminated all nematodes and most of the Pythiaceae fungi (Table 2). Midstudy assessments of ectomycorrhizae revealed Pt indices of 68 and <1 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae resembling those formed by Tt were found on 8 to 15 percent of short roots on control seedlings at that time.

At termination of the study, Virginia pine seedlings that received IMRD inoculum had a Pt index of 80; Abbott inoculum was ineffective (Table 3). Pt ectomycorrhizae formed by IMRD inoculum significantly increased seedling growth and significantly reduced culls in comparison to control seedlings. Seedling density averaged a very high 503 seedlings/m<sup>2</sup> and was not affected by treatment. Eight fruit bodies of Pt were produced in plots of IMRD inoculum, but they were not measured. Fruit bodies of other ectomycorrhizal fungi were not observed. The naturally occurring ectomycorrhizae resembled those formed by Tt.

In longleaf pine plots, soil fumigation eliminated all nematodes but only about half of the initially high population of Pythiaceae fungi (Table 2). Midstudy assessments of ectomycorrhizae revealed Pt indices of 18 and <1 for IMRD and Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae occurred on 6 to 11 percent of short roots on control seedlings at that time.

At termination of the study, longleaf pine seedlings that received IMRD inoculum had a Pt index of 54; Abbott inoculum was ineffective (Table 3). Inoculum treatments did not affect either seedling growth or number of culls in comparison to control seedlings. Seedling density varied from 5 to 237 seedlings/m<sup>2</sup> (average of 93 seedlings/m<sup>2</sup>) and was not affected by treatment. Fruit bodies of ectomy-

15 cm apart, of thiram-treated seeds of loblolly pine (120 seed/microplot) on May 6, 1977. The 50 oak plots of identical treatments were planted on May 7, 1977, with nontreated acorns of northern red oak. Acorns were planted 10 cm apart in each row for a total of 15 per microplot. Acorns were collected in October 1976 from select trees of northern red oak in the Cherokee National Forest, Tennessee, and stored at 5°C until planted. Seeds were covered with a 1-cm layer of fumigated pine straw. IMRD inoculum batch 5 was stored for 7 days and Abbott inoculum batch 2 was stored for 18 days before use. Midstudy evaluations were done in early August on four pine seedlings and two oak seedlings per plot. In mid-November, roots of all seedlings were cut vertically between rows and undercut with a shovel before hand lifting for evaluation. Seven oak seedlings per plot were evaluated. Pine seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 3 mm; oak seedlings were considered culls if shorter than 20 cm.

*Results:* Proper soil fumigation eliminated all organisms assayed but improper fumigation did not (Table 2). Midstudy assessments of ectomycorrhizae on the loblolly pine seedlings revealed Pt indices of 93, 63, 34, and 8 in properly fumigated soil and 86, 61, 18, and 3 in improperly fumigated soil for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae on control seedlings were observed on 29 to 36 percent and 39 to 51 percent of short roots in properly and improperly fumigated soil, respectively, at that time.

At termination of the study, pine seedlings that received IMRD and Abbott inocula at all rates produced high Pt indices (72 to 92) in properly fumigated soil and significantly increased seedling growth in comparison to control seedlings and seedlings from most treatments in improperly fumigated soil (Table 4). Improper soil fumigation reduced effectiveness of Abbott inoculum on loblolly pine from Pt indices of 90, 87, and 72 to 64, 34 and 7, respectively. Totals of 12, 5, 2, and 1 Pt fruit bodies were produced in properly fumigated soil and 17, 4, 2, and 1 in improperly fumigated soil by IMRD and the three decreasing rates of Abbott inocula, respectively. Seedling density averaged 123 seedlings/m<sup>2</sup> and was not affected by treatments. In improperly fumigated soil, Pt indices for IMRD and the high rate of Abbott inoculum on loblolly pine were >50. All rates of Abbott inoculum were significantly less effective in improperly fumigated soil than in properly fumigated soil. IMRD inoculum significantly reduced culls by more than half in improperly fumigated soil in comparison to other treatments. In a comparison of the same inoculum treatments in properly and improperly fumigated soil, pine seedlings in the properly fumigated soil were consistently larger and had significantly fewer culls.

Midstudy assessments of ectomycorrhizae on the oak seedlings revealed Pt indices of 73, 12, 2, and 5 in properly fumigated soil and 48, 7, 2, and 0 in improperly fumigated soil for IMRD and the three decreasing rates of Abbott inocula, respectively.

At termination of the oak seedling study, those that received IMRD and the highest rate of Abbott inoculum had Pt indices of 76 and 68, respectively, in properly fumigated soil. Seedling growth was significantly increased by Pt ectomycorrhizae and number of culls were reduced by all treatments except the low rate of Abbott inoculum in comparison to control seedlings (Table 4). In improperly fumigated soil, seedlings that received IMRD inoculum had a Pt index of 53, but Pt indices for the Abbott inoculum were only between 6 and 24. Pt ectomycorrhizae formed by IMRD inoculum significantly increased oak seedling growth and significantly reduced culls by nearly a factor of 3 in comparison to control seedlings. Improper soil fumigation did not strongly affect oak seedling growth but significantly reduced effectiveness of all inoculum treatments as mea-

TABLE 4. Growth and ectomycorrhizal development of loblolly pine and northern red oak seedlings in properly and improperly fumigated soil with vegetative inoculum of *Pisolithus tinctorius* (Pt) produced by the Institute for Mycorrhizal Research and Development (IMRD) and batch 2 produced by Abbott Laboratories in 1977.<sup>1</sup>

Tree species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ectomycor- rhizal with—		Per- cent seed- lings with Pt	Pt in- dex <sup>2</sup>	Per- cent cull seed- lings
			Top	Root	Total	Pt	All fungi			
Loblolly pine:										
proper fumigation										
IMRD 1.08	22.1a	4.7a	8.9a	4.0a	12.9a	51a	56a	100a	92c	9a
Abbott 1.62	22.3a	4.6ab	9.1a	3.8a	12.9a	44a	49a	100a	90a	12a
Abbott 1.08	23.0a	4.6ab	8.9a	3.7a	12.6a	41a	47a	100a	87a	16a
Abbott 0.54	21.3ab	4.4b	8.8a	3.4ab	12.2a	26b	35ab	100a	72a	10a
Control	19.7b	4.4b	7.7b	3.0b	10.7b	0	17b	0	0	5a
improper fumigation										
IMRD 1.08	21.2a	4.3a	7.1ab	3.3a	10.4ab	40a	45ab	98a	87a	11b
Abbott 1.62	21.0a	4.3a	7.3ab	3.2ab	10.5ab	36a	50a	92b	64b	28a
Abbott 1.08	21.1a	4.4a	7.2ab	3.3a	10.4ab	24b	49ab	76c	34c	24ab
Abbott 0.54	21.5a	4.4a	7.7a	3.4a	11.1a	9c	43b	32d	7d	17ab
Control	19.1b	4.0b	6.7b	3.0b	9.6b	0d	39b	0	0	29a
Northern red oak:										
proper fumigation										
IMRD 1.08	34.8a	7.8a	13.0b	17.0ab	30.0a	26a	33a	100a	76a	7a
Abbott 1.62	35.7a	7.8a	15.2a	19.2a	34.4a	18ab	27ab	100a	68b	3a
Abbott 1.08	33.9ab	7.3ab	12.4b	15.0b	27.4b	13ab	25ab	91a	46c	7a
Abbott 0.54	37.1a	7.4ab	13.2ab	16.4ab	29.6ab	10b	23ab	74b	33d	12b
Control	31.7b	6.9b	12.5b	15.6b	28.1b	0	17b	0	0	14b
improper fumigation										
IMRD 1.08	40.2a	7.7a	15.2a	18.9a	34.1a	17a	31a	100a	53a	7c
Abbott 1.62	32.5b	7.2b	12.1b	15.3b	27.4b	9b	29a	86b	24b	20b
Abbott 1.08	32.1b	7.5ab	11.7bc	16.1ab	27.8b	8b	26a	63b	19b	17b
Abbott 0.54	30.9b	7.1b	10.3c	15.3b	25.6c	4c	27a	37c	6c	18b
Control	32.1b	6.8b	10.4c	14.8b	25.2c	0	26a	0	0	26a

<sup>1</sup> Means in a tree species sharing a common letter in the same soil fumigation treatment but between inoculum treatments are not significantly different at  $P = 0.05$ . Underlined means in a tree species in the same inoculum treatment but between different soil fumigation treatments are significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

sured by Pt index. Seedling density averaged 31 seedlings/m<sup>2</sup> and was not affected by treatment. Totals of 15, 5, 3, and 0 fruit bodies of Pt were produced in properly fumigated soil and 6, 1, 0, and 0 fruit bodies in improperly fumigated soil by IMRD and the three decreasing rates of Abbott inocula, respectively. Most of the

naturally occurring ectomycorrhizae detected on the pine and oak seedlings, regardless of treatment, resembled those formed by Tt. Fruit bodies of Tt occurred on more than 15 percent of all sampled pine and oak seedlings. More Tt ectomycorrhizae occurred on pine and oak seedlings in improperly fumigated soil than in properly fumigated soil.

## DISCUSSION

Abbott batches 1 and 2 were not consistently effective in forming Pt ectomycorrhizae and failed to produce Pt indices  $>50$  in most tests. Only with loblolly pine at the Great Southern Nursery using batch 1 and at the IMRD Microplot Nursery on loblolly pine and northern red oak using batch 2 did the Pt indices of Abbott inocula meet minimum inoculum standards. Abbott batch 2 was also ineffective in container-grown seedling tests on various pine species at the IMRD and in North Carolina and North Dakota (Marx and others 1982).

Length of storage of Abbott inoculum before use did not appear to be related to its effectiveness. Storage also did not alter efficacy of IMRD inoculum, since it was stored from 1 to 22 days in these tests without apparent loss of ability to form ectomycorrhizae.

Lack of adequate soil fumigation did not appear to explain the results either. Both batches of Abbott inoculum were ineffective regardless of effectiveness of soil fumigation. The exception to this was in the IMRD Microplot Nursery. Results of this test in improperly fumigated soil showed that proper soil fumigation is very important to inoculum effectiveness. However, even in improperly fumigated soil, Abbott inoculum batch 2 was more effective on pine at the IMRD nursery than at any conventional nursery. Perhaps soil conditions in IMRD microplots are more conducive to Pt ectomycorrhizal development than in conventional nurseries.

Other cultural factors such as soil fertility, use of pesticides, previous cover crops, and seedling density also were not related to inoculum effectiveness. In most nurseries, IMRD inoculum, which received the same cultural practices as Abbott inoculum, produced very high Pt indices and numerous fruit bodies. This showed that effective inoculum could form ectomycorrhizae under a variety of soil and cultural conditions encountered in these nurseries.

IMRD inoculum at the two Weyerhaeuser nurseries did not produce Pt indices  $>50$  on loblolly pine seedlings either at midstudy (average 37) or at final assessments (average 30). We cannot explain this because, with the exception of the herbicide napropamide, cultural practices at these nurseries were similar to those at other nurseries. Low Pt indices at midstudy, however, indicate the problem existed early in the study. Even though acceptable Pt indices were not obtained in these nurseries, significant increases in seedling fresh weights in the new section in the Oklahoma nursery and a significant reduction in percent culls in the old section in Oklahoma and in the Arkansas nursery were observed between seedlings in the IMRD inoculum plots and control plots. During 2 years of earlier tests at the Oklahoma nursery, IMRD vegetative inoculum was highly effective in forming Pt ectomycorrhizae on loblolly pine seedlings in which similar cultural practices, but without napropamide, were used (Marx and others 1978, 1979).

The favorable results with IMRD inoculum in the 2-0 ponderosa pine test at the Bessey Tree Nursery indicated that effective Pt inoculum not only significantly increases seedling growth and significantly reduces the number of culls, but also persists in the nursery soil for two growing seasons and two winter periods without significant supplantation by indigenous ectomycorrhizal fungi.

Abundant Pt ectomycorrhizal development from IMRD inoculum significantly increased seedling growth (total fresh weight) in 11 of the 21 nursery tests and significantly reduced culls in 14 of the 21 tests undertaken in 1977. Since seedling

tops were mowed to a height of 20 to 25 cm at least once during the growing season in several southern nurseries, true height and foliar weight measurements could not be obtained. Growth differences in these nurseries, therefore, may have been greater than those reported here.

High Pt indices were associated consistently with large numbers of Pt fruit bodies. Fruit bodies found in plots of both IMRD and Abbott inocula were either sessile or stalked and ranged in size from 1 to 15 cm in diameter. Frequently, broad variations in shape and size were observed within the same test plot at the same collection period. Since shape and size of fruit bodies are highly variable within the same isolate of Pt these characteristics do not appear reliable in identifying biotypes of the fungus.

Results of midstudy assessments of ectomycorrhizae on the test seedlings correlated well with results obtained at the end of these tests. In the 16 tests with 1-0 pine seedlings in conventional nurseries in 1977, Pt indices from IMRD inoculum obtained at midstudy averaged 54 and at final evaluation they averaged 64. Although midstudy assessments slightly underestimated the final Pt indices in these tests, results indicated that midstudy assessments can be used to predict inoculum effectiveness and the incidence of competitive, naturally occurring ectomycorrhizal fungi.

Tt was the most frequently encountered naturally occurring ectomycorrhizal fungus. Fruit bodies of this fungus were found consistently on seedlings in all nurseries, however, it was more plentiful in the southern nurseries. The Tt ectomycorrhizae were macroscopically identical to those described earlier on various pines (Marx and Bryan 1970). Fruit bodies of *R. nigrescens* were observed in abundance (6 to 10/m<sup>2</sup> in nursery soil) in the Buckeye and Andrews Nurseries in Florida and in the Great Southern Nursery in Georgia. Numerous white hyphal strands of this fungus connected the fruit bodies to the pure white, complex coralloid ectomycorrhizae. Although this fungus is widely distributed on pines in the tropics (Ivory 1980), this is the first report of the association of this fungus with ectomycorrhizae of pines in the United States.

Since the 1977 Abbott inoculum was not effective, procedures for producing and processing this commercial inoculum were modified during late 1977 and early 1978 in an attempt to improve its quality. An expanded trial was implemented in 1978 at additional nursery locations to ascertain the effectiveness of different batches of Abbott inoculum.

## 1978 TESTS

### MATERIALS AND METHODS

Three batches of IMRD inoculum were produced as in 1977. Three batches of Abbott inoculum, unlike those of 1977, were grown in fermentation trays containing vermiculite and 10 percent peat moss by volume. After steaming, the substrate was inoculated with starter mycelium, harvested, and dried as in 1977. Inoculum was not leached. Physical characteristics of inoculum used in 1978 are shown in Table 5. Inoculum from both sources was packed and transported to test nurseries as in 1977. Plot designs, installation, and monitoring procedures used in the 1977 tests were followed in 1978. The rates of Abbott inoculum used in the 1978 tests were 2.16, 1.08, and 0.54 l/m<sup>2</sup> and IMRD inoculum was tested at a rate of 1.08 l/m<sup>2</sup>. Chemical analyses of soil were made as in 1977 except that amounts of organic matter were not determined.

### NURSERY INFORMATION AND RESULTS

Cropping history and cultural practices involved in the 1978 nursery tests prior to study installation are given in Appendix IV, chemical and physical character-

TABLE 5. Physical characteristics and ability of vegetative inocula of *Pisolithus tinctorius* (Pt) produced in 1978 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories to form ectomycorrhizae and fruit bodies on loblolly pine seedlings after 4 and 8 months in microplots at the IMRD.<sup>1</sup>

Inoculum source	Bulk density (g/l)	Moisture content (percent)	Storage at 5°C (days)	Pt index <sup>2,3</sup>		Number Pt fruit bodies after 8 months
				4 months	8 months	
IMRD						
Batch 2	318	15.8	21	94a	82a	8
Batch 4	320	7.6	1	89a	83a	11
Batch 5	337	18.2	1	94a	90a	9
Abbott						
Batch 2	241	25.8	15	1d	1c	0
Batch 4	226	28.5	3	14c	18b	0
Batch 5	230	28.2	3	79b	82a	5

<sup>1</sup> Approximately 45 seedlings from each of five replicate microplots were assessed for ectomycorrhizal development after 4 and 8 months for each of the six inoculum treatments.

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> Pt indices in a column followed by a common letter are not significantly different at  $P = 0.05$ .

istics of soil at study installation are given in Appendix V, and cultural practices involved after study installation are shown in Appendix VI; other details are as follows:

*IMRD Microplot Nursery, Athens, Georgia.*—Since only one batch each of IMRD and Abbott inoculum was to be tested in a given conventional nursery, all inoculum batches were tested under identical conditions at the IMRD Microplot Nursery so that more reliable comparisons between inoculum sources and batches could be made. Five replicate microplots were installed for each of the six inocula (each tested at the rate of 1.08 l/m<sup>2</sup>). Fumigation was done following procedures used in 1977 for properly fumigated soil. After fertilizer additions and inoculum mixing in April 1978, thiram-treated seeds of loblolly pine were planted in each microplot in four rows spaced 12 cm apart. Two rows of seedlings in each microplot were lifted in August after 4 months for midstudy root assessments and the remaining two rows of seedlings were lifted in December after 8 months. Seedlings were assessed only for ectomycorrhizal development.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Assessment of seedlings after 4 and 8 months showed that all IMRD inocula were effective and produced Pt indices above 82 after 8 months (Table 5). Batch 5 of Abbott inoculum also produced a Pt index of 82 at 8 months but batches 2 and 4 produced Pt indices of only 1 and 18, respectively. Storage of IMRD inoculum for up to 21 days and drying to less than an 8 percent moisture content did not alter efficacy. Pt indices of each batch of IMRD inoculum were similar after 4 and 8 months of seedling development.

*Great Southern Paper Company Nursery, Georgia.*—Soil conditions during fumigation were similar to those in 1977. Batch 2 inocula of IMRD and Abbott were stored for 6 and 4 days, respectively, before use. Inoculum was applied and nontreated seeds of loblolly pine were planted on April 11, 1978. Seeds were

TABLE 6. Numbers of Pythiaceae fungal propagules (pg) and plant parasitic nematodes isolated from pre- and post-fumigation soil samples in nursery tests in 1978.

Nursery	Time of fumigation	Pythiaceae fungi <sup>1</sup> pg/g		Nematodes/475 cc soil	
		Pre-fum	Post-fum	Pre-fum	Post-fum
IMRD, GA	Spring 1978	39	0	36 <sup>2,3,4</sup>	0
Great Southern, GA	Spring 1978	27	4	67 <sup>2,3,5</sup>	0
Westvaco, SC	Spring 1978	1	0	183 <sup>4,6,7</sup>	0
Kimberly-Clark, AL	Spring 1978	2	0	0 <sup>2</sup>	0
Weyerhaeuser, OK	Spring 1978	6	0	0 <sup>2</sup>	0
Weyerhaeuser, AR	Spring 1978	1	0	0 <sup>2</sup>	0
Waynesboro, MS	Spring 1978	12	0	0 <sup>2</sup>	0
Buckeye, FL	Spring 1978	2	0	0 <sup>2</sup>	0
Beauregard, LA	Spring 1978	45	6	363 <sup>2,6</sup>	8 <sup>2,6</sup>
Union State, IL	Fall 1977	1	0	0 <sup>2</sup>	0
George O. White, MO	Fall 1977	0	0	0 <sup>2</sup>	0
Vallonia, IN	Spring 1978	6	0	0 <sup>2</sup>	0
Tyee Tree Farm, OR	Fall 1977	61	1	150 <sup>3,5,6</sup>	0
Coeur d'Alene, ID	Summer 1977	—	0	—	0
Parsons State, WV	Fall 1977	0	2	0	0 <sup>2</sup>
Marietta State, OH	Fall 1977	—	7	—	0 <sup>2</sup>
Oklahoma State, OK	Spring 1978	8	0	0 <sup>2</sup>	0
New Kent, VA	Spring 1978	6	0	0 <sup>2</sup>	0
Indust. For. Assoc., WA	Fall 1977	—	2	—	0 <sup>2</sup>
Bessey, NE	Spring 1978	35	0	52 <sup>2,7</sup>	0 <sup>2</sup>
Edwards, NC	Fall 1977	—	3	—	0
Potlatch, MN	Spring 1978	14	1	0 <sup>2</sup>	0 <sup>2</sup>
NEPCO, WI	Spring 1978	58	0	0	0
USDA-SCS, MI	Fall 1977	—	3	—	0 <sup>2</sup>
Toumey, MI	Fall 1977	—	0	0	0 <sup>2</sup>

<sup>1</sup> Mainly *Pythium irregulare*.

<sup>2</sup> Saprophytic nematodes.

<sup>3</sup> Dagger nematode (*Xiphinema*).

<sup>4</sup> Stunt nematode (*Tylenchorhynchus*).

<sup>5</sup> Lesion nematode (*Pratylenchus*).

<sup>6</sup> Ring nematode (*Criconeoides*).

<sup>7</sup> Stubby root (*Trichodorus*).

covered with 1,560 kg/ha of hydromulch. Midstudy root assessments were made in August, and the study was terminated in January 1979. Characteristics of cull seedlings were as in 1977.

**Results:** Soil fumigation eliminated all but a few Pythiaceae fungi (Table 6). Two days after study installation, beds were reshaped and reseeded due to damage by heavy rains. Midstudy assessment of ectomycorrhizae on the seedlings revealed Pt indices of 91 and 0 for IMRD and Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on 7 to 12 percent of short roots on control seedlings; a few Tt fruit bodies were observed at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 70; Abbott inoculum formed a trace of Pt ectomycorrhizae (Table 7). Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth and reduced culls by nearly half in comparison to control seedlings. All rates of Abbott inoculum increased culls by an average of 43 percent. Seedling density

TABLE 7. Growth and ectomycorrhizal development of pine and Douglas-fir seedlings in various nurseries with vegetative inoculum of *Pisolithus tinctorius* (Pt) produced in 1978 by the Institute for Mycorrhizal Research and Development (IMRD) and batches 2, 4, and 5 produced by Abbott Laboratories.<sup>1</sup>

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Great Southern, GA										
loblolly pine										
IMRD 1.08	26.8a <sup>3</sup>	5.4a	14.0a	6.9a	20.9a	43a	56a	100a	71a	14c
Abbott #2, 2.16	22.1b	4.4b	8.6b	4.4b	13.0b	1b	38b	6b	< 1b	33a
Abbott #2, 1.08	22.5b	4.0b	7.1b	3.2b	10.3b	3b	34b	16b	1b	30a
Abbott #2, 0.54	23.4ab	4.5b	9.1b	4.8b	13.9b	6b	38b	22b	3b	36a
Control	24.0ab	4.2b	7.7b	3.4b	11.1b	0	39b	0	0	23b
Westvaco, SC										
loblolly pine										
IMRD 1.08	26.6a <sup>3</sup>	9.5a	31.8a	19.1a	51.9a	49a	68a	100a	71a	6b
Abbott #2, 2.16	26.6a	8.2b	23.8b	15.5ab	39.3b	3b	64ab	14b	<1b	15ab
Abbott #2, 1.08	24.2a	7.7b	20.3b	12.2b	32.5b	0	65ab	0	0	13ab
Abbott #2, 0.54	25.0a	7.6b	19.1b	11.8b	30.9b	0	55b	0	0	12ab
Control	26.6a	7.9b	21.4b	13.0b	34.4b	0	61ab	0	0	17a
Kimberly-Clark, AL										
loblolly pine										
IMRD 1.08	29.1a <sup>3</sup>	6.1a	16.9a	7.0a	23.9a	47a	55a	100a	89a	12b
Abbott #2, 2.16	28.1a	5.9a	15.7a	5.6b	21.3ab	10b	43b	40b	9b	17ab
Abbott #2, 1.08	31.5a	6.0a	16.6a	5.8ab	22.4ab	3b	41b	26b	2b	10b
Abbott #2, 0.54	29.8a	6.0a	16.2a	5.5b	21.7ab	6b	44b	20b	4b	17ab
Control	29.2a	5.5a	14.3a	5.2b	19.5b	0	41b	0	0	24a
Weyerhaeuser, OK										
loblolly pine										
IMRD 1.08	23.6a <sup>3</sup>	5.4a	14.9a	4.4a	19.3a	16a	22a	100a	70a	26b
Abbott #2, 2.16	17.5b	4.2b	8.0c	2.9b	10.9c	2b	17b	12b	1b	49a
Abbott #2, 1.08	20.4ab	4.2b	8.3c	2.5b	10.8c	1b	15b	8b	<1b	37b

TABLE 7. Continued.

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Abbott #2, 0.54	23.1a	4.6b	9.8bc	2.9b	12.7bc	1b	15b	4b	<1b	33b
Control	24.1a	4.8b	11.7b	3.0b	14.7b	0	18b	0	0	27b
Weyerhaeuser, AR										
loblolly pine										
IMRD 1.08	22.3a <sup>3</sup>	4.7ab	11.6ab	2.5ab	14.1ab	9a	16b	78a	41a	22a
Abbott #2, 2.16	22.5a	4.5bc	10.3bc	2.3b	12.6ab	0	22ab	0	0	26a
Abbott #2, 1.08	20.2a	4.3c	8.8c	2.0c	10.8c	0	19ab	0	0	26a
Abbott #2, 0.54	22.0a	4.9ab	11.4ab	2.6ab	14.0ab	1b	26a	8b	<1b	23a
Control	23.2a	5.1a	13.5a	2.9a	16.4a	0	21ab	0	0	20a
Waynesboro, MS										
loblolly pine										
IMRD 1.08	25.3a <sup>3</sup>	5.7a	20.5a	5.7ab	26.2a	28a	50a	100a	57a	18b
Abbott #2, 2.16	23.2a	5.3ab	16.3b	4.8b	21.1ab	7b	41b	48b	8b	28ab
Abbott #2, 1.08	24.0a	5.1c	15.3b	4.5b	19.8b	2b	33c	16c	1b	23ab
Abbott #2, 0.54	23.8a	5.8a	17.0ab	6.2a	23.2ab	3b	44ab	22c	1b	29ab
Control	22.8a	5.4ab	16.0b	5.5ab	21.5ab	4b	32c	20c	3b	39a
Buckeye, OH										
slash pine										
IMRD 1.08	27.3ab <sup>3</sup>	4.2a	10.1a	2.2a	12.3a	16a	34a	94a	43a	7a
Abbott #2, 2.16	28.3a	4.4a	10.4a	2.0a	12.4a	2b	29ab	14b	1b	9a
Abbott #2, 1.08	27.7ab	4.3a	10.2a	2.1a	12.3a	2b	27b	12b	1b	12a
Abbott #2, 0.54	26.3b	4.5a	10.9a	2.4a	13.3a	2b	27b	24b	2b	14a
Control	27.0ab	4.5a	11.6a	2.3a	13.9a	<1b	27b	2c	<1b	13a
Beauregard, LA										
longleaf pine										
IMRD 1.08	—	10.5a	27.1a	12.5a	39.6a	13a	16a	100a	83a	57a
Abbott #2, 2.16	—	9.5b	18.4b	10.3b	28.7b	1b	14a	8b	<1b	57a

TABLE 7. *Continued.*

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Abbott #2, 1.08	—	10.1ab	20.2b	10.4b	30.6b	1b	15a	6b	<1b	58a
Abbott #2, 0.54	—	10.0ab	19.5b	10.7b	30.2b	0	15a	0	0	64a
Control	—	10.2a	20.0b	10.2b	30.2b	0	13a	0	0	66a
Union State, IL loblolly pine										
IMRD 1.08	24.9a <sup>3</sup>	5.7a	13.4a	4.2a	17.6a	17a	36a	80a	40a	13a
Abbott #4, 2.16	24.7a	5.6a	12.9a	3.9a	16.8a	8b	27a	56ab	17b	14a
Abbott #4, 1.08	25.2a	5.3a	12.3a	3.7a	16.0a	7b	30a	56ab	13b	13a
Abbott #4, 0.54	23.8a	5.4a	12.3a	3.3a	15.6a	4c	27a	28bc	4c	11a
Control	24.5a	5.1a	10.9a	3.3a	14.2a	0	28a	0	0	13a
George O. White, MO shortleaf pine										
IMRD 1.08	14.3a	3.3a	3.6a	1.9a	5.5a	20a	39a	88a	46a	27a
Abbott #4, 2.16	14.5a	3.3a	3.8a	2.0a	5.8a	3b	38a	32b	3b	23a
Abbott #4, 1.08	15.2a	3.3a	3.8a	2.0a	5.8a	3b	34a	34b	3b	29a
Abbott #4, 0.54	15.2a	3.5a	4.1a	2.2a	6.3a	3b	32a	26b	2b	35a
Control	14.4a	3.4a	3.9a	2.1a	6.0a	0	30a	0	0	29a
Vallonia, IN Virginia pine										
IMRD 1.08	21.7a	4.5a	15.3a	9.8a	25.0a	35a	40a	100a	89a	11a
Abbott #4, 2.16	21.4a	4.1a	11.0b	7.3b	18.3b	25a	30ab	100a	81a	10a
Abbott #4, 1.08	21.6a	4.3a	12.4ab	7.8ab	20.2ab	5b	15c	46b	14b	8a
Abbott #4, 0.54	20.8a	4.3a	11.7b	8.9ab	20.6ab	11b	22bc	60b	30b	13a
Control	20.8a	4.5a	12.9ab	8.9ab	21.8ab	0	19bc	0	0	14a
Tyee Tree Farm, OR Douglas-fir (2-0)										
IMRD 1.08	24.1a	6.0a	15.2a	9.1a	24.3a	1	41a	5	<1	ND
Abbott #4, 2.16	25.8a	6.3a	16.4a	8.7a	25.1a	0	33b	0	0	ND

TABLE 7. *Continued.*

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Abbott #4, 1.08	25.5a	5.7a	14.7a	9.2a	23.9a	0	38ab	0	0	ND
Abbott #4, 0.54	26.5a	5.2a	12.9a	7.0a	19.9a	0	23c	0	0	ND
Control	21.8a	5.3a	11.5a	6.7a	18.3a	0	34ab	0	0	ND
Coeur d'Alene, ID Douglas-fir (2–0)										
IMRD 1.08	23.1ab	4.6ab	6.9ab	6.0ab	12.9ab	0	32ab	0	0	ND
Abbott #4, 2.16	25.0a	4.8a	9.5a	7.1a	16.6a	0	36a	0	0	ND
Abbott #4, 1.08	20.8b	4.1ab	5.8b	5.6ab	11.4b	0	21c	0	0	ND
Abbott #4, 0.54	21.7ab	3.9b	5.6b	4.9b	10.5b	0	26bc	0	0	ND
Control	22.9ab	4.3ab	6.7ab	5.5ab	12.2ab	0	22c	0	0	ND
Parsons, WV eastern white pine (2–0)										
IMRD 1.08	13.2a	4.1a	7.1a	4.9a	12.0a	18a	37a	96a	47a	36a
Abbott #4, 2.16	13.6a	3.7a	5.6a	3.8a	9.4a	1b	28b	6b	<1b	43a
Abbott #4, 1.08	12.5a	3.9a	5.9a	4.9a	10.8a	0	28b	0	0	47a
Abbott #4, 0.54	12.9a	3.8a	5.7a	4.1a	9.8a	0	27b	0	0	43a
Control	13.1a	3.8a	6.3a	4.1a	10.4a	0	28b	0	0	46a
Marietta, OH eastern white pine (2–0)										
IMRD 1.08	18.5b	5.2ab	10.4ab	6.5ab	16.9a	7a	32a	54a	12a	24a
Abbott #4, 2.16	18.1b	5.4ab	10.8ab	7.4a	18.2a	1b	30a	6b	<1b	25a
Abbott #4, 1.08	18.8b	5.2ab	9.9bc	6.4b	16.3ab	1b	31a	5b	<1b	24a
Abbott #4, 0.54	18.3b	5.0b	9.1c	6.0b	15.1b	1b	29a	2b	<1b	20a
Control	20.7a	5.6a	12.8a	7.0ab	19.8a	0	22b	0	0	22a
Oklahoma State, OK Austrian pine (2–0)										
IMRD 1.08	18.9a	8.7a	33.3a	16.3ab	49.6a	4a	17a	56a	14a	25b

TABLE 7. Continued.

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Abbott #4, 2.16	16.3ab	8.7a	33.4a	20.1a	53.5a	3a	18a	22bc	4b	27b
Abbott #4, 1.08	18.1a	8.6a	33.2a	16.9a	50.1a	3a	20a	30ab	5b	23b
Abbott #4, 0.54	16.5ab	8.1ab	27.4ab	15.3ab	42.7ab	4a	18a	42ab	9b	25b
Control	13.4b	7.2b	19.5b	11.2b	30.7b	0	13b	0	0	41a
New Kent, VA										
loblolly pine										
IMRD 1.08	17.3a <sup>1</sup>	3.3a	4.1a	2.0a	6.1a	41a	49a	100a	86a	9a
Abbott #5, 2.16	16.8a	3.1a	3.6ab	1.7a	5.3a	6b	39ab	56b	9b	9a
Abbott #5, 1.08	16.7a	3.0a	3.2ab	1.7a	4.9a	3b	34b	32c	3b	10a
Abbott #5, 0.54	17.1a	3.0a	3.0b	1.6a	4.6a	4b	42ab	18d	2b	11a
Control	16.9a	3.3a	4.1a	1.8a	5.9a	0	46a	0	0	13a
Industrial Forest, WA										
Douglas-fir (2–0)										
IMRD 1.08	31.9a	3.5a	7.0a	3.0a	10.0a	0	33ab	0	0	ND
Abbott #5, 2.16	33.4a	3.4a	6.0a	2.8a	8.8a	0	49a	0	0	ND
Abbott #5, 1.08	32.2a	3.9a	6.5a	2.8a	9.3a	0	49a	0	0	ND
Abbott #5, 0.54	33.0a	3.5a	5.3a	2.5a	7.8a	0	48a	0	0	ND
Control	30.3a	3.4a	5.5a	2.2a	7.7a	0	35b	0	0	ND
Bessey, NE										
Austrian pine (2–0)										
IMRD 1.08	25.2a	7.3a	28.9a	9.3a	38.2a	12a	36a	92a	33a	34a
Abbott #5, 2.16	24.6a	6.9ab	27.8a	8.9a	36.7a	12a	41a	74b	23b	33a
Abbott #5, 1.08	25.5a	6.2b	27.3a	8.5a	35.8a	13a	42a	58b	18b	33a
Abbott #5, 0.54	25.8a	6.2b	27.0a	8.3a	35.3a	8a	36a	68b	16b	35a
Control	24.4a	6.9ab	28.9a	8.8a	37.7a	0	33a	0	0	32a

TABLE 7. *Continued.*

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Edwards, NC										
eastern white pine (2–0)										
IMRD 1.08	18.8a	5.7a	12.3a	10.9a	23.2a	19a	39a	96a	48a	19c
Abbott #5, 2.16	17.2ab	5.6a	11.0ab	11.7a	22.7a	14ab	36a	92a	35ab	27b
Abbott #5, 1.08	17.2ab	5.4a	10.2bc	10.4a	20.6ab	9b	36a	66b	18b	31ab
Abbott #5, 0.54	15.9bc	5.3a	9.1c	9.4a	18.5b	9b	33a	54b	13b	35ab
Control	15.0c	5.3a	8.8c	10.1a	18.9b	1c	37a	22c	<1c	41a
Potlatch, MN										
red pine (2–0)										
IMRD 1.08	5.5a	3.3a	5.9a	2.7a	8.6a	15a	36a	98a	42a	19a
Abbott #5, 2.16	4.4b	3.1ab	5.3b	2.3ab	7.6b	1b	32ab	18bc	<1b	22a
Abbott #5, 1.08	5.4a	3.0b	5.6ab	2.3ab	7.9ab	2b	29b	22b	2b	20a
Abbott #5, 0.54	4.6b	2.7c	4.4c	1.8c	6.2c	1b	34ab	6c	<1b	21a
Control	4.7b	3.1b	4.5c	2.1bc	6.6c	0	27b	0	0	24a
NEPCO, WI										
red pine (2–0)										
IMRD 1.08	17.5a	3.5a	8.4ab	2.2b	10.6a	7a	32a	78a	15a	30b
Abbott #5, 2.16	18.2a	3.5a	9.3a	2.3b	11.6a	6a	29ab	60a	12a	32b
Abbott #5, 1.08	17.0a	3.6a	9.3a	2.7a	12.0a	4a	32a	28b	4b	37b
Abbott #5, 0.54	15.2a	3.5a	7.6b	2.2b	9.8a	4a	26b	55ab	9b	32b
Control	15.4a	3.4a	7.8ab	2.3b	10.1a	0	27b	0	0	49a
USDA-SCS, MI <sup>4</sup>										
red pine (2–0)										
IMRD 1.08	13.6	3.9	10.3	2.7	13.0	36	51	100	72	16
Abbott #5, 2.16	14.8	4.1	11.5	3.1	14.6	6	34	60	10	11
Abbott #5, 1.08	12.9	3.8	10.5	2.6	13.1	10	33	60	18	9
Abbott #5, 0.54	13.2	4.1	10.1	3.0	13.1	2	34	25	2	18
Control	14.1	4.1	10.6	2.5	13.1	0	46	0	0	13

TABLE 7. *Continued.*

Location, species, Pt inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Stem dia. (mm)	Fresh weight (g)			Percent short roots ecto- mycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent cull seedlings
			Top	Root	Total	Pt	All fungi			
Toumey, MI red pine (3–0)										
IMRD 1.08	23.1a	4.8a	21.5a	6.2a	27.7a	4a	47a	18a	2a	17b
Abbott #5, 2.16	22.4a	4.5a	19.7a	4.8b	24.5a	2a	46a	5b	1b	21ab
Abbott #5, 1.08	22.3a	4.9a	19.3a	4.8b	24.1a	1a	41a	5b	< 1c	23a
Abbott #5, 0.54	21.4a	4.8a	17.9a	4.7b	22.6b	1a	41a	4b	< 1c	19b
Control	21.4a	4.7a	20.9a	5.4ab	26.3a	0	44a	0	0	26a

<sup>1</sup> Means sharing a common letter in the same nursery but between inoculum treatments are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> Seedling tops mowed to approximately 20 cm at least once during growing season.

<sup>4</sup> Only two blocks lifted and evaluated. Three blocks inadvertently lifted by nursery personnel; statistical comparisons were not made.

averaged 380/m<sup>2</sup> and was not affected by treatment. Eight fruit bodies of *Pt* from IMRD inoculum plots and one from Abbott inoculum at the 0.54 l/m<sup>2</sup> rate were found. These ranged in size from 3 to 10 cm in diameter; all were stalked. One fruit body was 29 cm tall. Fruit bodies of *Tt* and *R. nigrescens* were abundant in all plots and each appeared equal in forming naturally occurring ectomycorrhizae.

*Westvaco Corporation Nursery, South Carolina.*—Soil conditions during fumigation were similar to those in the 1977 study. Batch 2 inocula of IMRD and Abbott were stored for 5 and 3 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 10, 1978. Seeds were covered with 1,680 kg/ha of hydromulch. Midstudy root assessments were made in August, and the study was terminated in January 1979. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed *Pt* indices of 86, 7, 3, and 2 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on 10 to 42 percent of short roots on control seedlings; fruit bodies were not detected at that time.

At termination of the study, seedlings that received IMRD inoculum had a *Pt* index of 71; Abbott inoculum was ineffective (Table 7). *Pt* ectomycorrhizae from IMRD inoculum significantly increased seedling growth by 51 percent and reduced culls by more than half in comparison to control seedlings. The Abbott inoculum did not affect seedling growth or number of culls. Inoculated plots, which had been chopped with hand tools to incorporate inoculum, had fewer seedlings (116/m<sup>2</sup>) than nonchopped control plots (150/m<sup>2</sup>). Two *Pt* fruit bodies (6 and 9 cm diameter, sessile) were found in IMRD inoculum plots and numerous *Tt* fruit bodies were recorded in all plots. Naturally occurring ectomycorrhizae resembled those formed by *Tt*.

*Kimberly-Clark Corporation Nursery, Alabama.*—Soil conditions during fumigation were similar to those in the 1977 study. Batch 2 inocula of IMRD and Abbott were stored for 21 and 19 days, respectively, before use. Inoculum was applied and treated (anthraquinone, captan, and aluminum oxide) seeds of loblolly pine were planted on April 26, 1978. Seeds were covered with a 1-cm layer of nonfumigated sawdust as a mulch. Midstudy root assessments were made in early August, and the study was terminated in early April 1979. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on seedlings revealed *Pt* indices of 77 and <1 for IMRD and Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on 15 to 44 percent of short roots; *Tt* fruit bodies were present in all plots at that time.

At termination of the study, seedlings that received IMRD inoculum produced a *Pt* index of 89; Abbott inoculum had an average *Pt* index of 5 (Table 7). *Pt* ectomycorrhizae from IMRD inoculum significantly increased seedling growth and reduced culls by half in comparison to control seedlings. Abbott inoculum did not affect seedling growth but significantly reduced culls at the 1.08 l/m<sup>2</sup> rate. Inoculated and chopped plots had fewer seedlings (350/m<sup>2</sup>) than nonchopped control plots (477/m<sup>2</sup>). Six *Pt* fruit bodies (3 to 11 cm diameter, stalked) were observed in IMRD inoculum plots; *Tt* fruit bodies were present in all plots. Naturally occurring ectomycorrhizae appeared to be formed primarily by *Tt*.

*Weyerhaeuser Company Nursery, Oklahoma.*—Soil conditions during fumigation were similar to those in 1977. Batch 2 inocula of IMRD and Abbott were stored for 27 and 25 days, respectively, before use. Inoculum was applied and thiram-

treated seeds of loblolly pine were planted on May 2, 1978. Seeds were covered with 1,680 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in February 1979. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation eliminated the low populations of organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 60, 9, 3, and 2 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on 2 to 9 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 70; Abbott inoculum was ineffective (Table 7). Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth but did not affect number of culls in comparison to control seedlings. The highest rate of Abbott inoculum increased culls by nearly half and seedlings in the two highest rates were smaller than control seedlings. Seedling density averaged 376/m<sup>2</sup> and was not affected by treatment. Fruit bodies of Pt were not detected and those of Tt were rare. Incidence of naturally occurring ectomycorrhizae was low, and they resembled those formed by Tt.

*Weyerhaeuser Company Nursery, Arkansas.*—Soil conditions during fumigation were similar to those in 1977. Batch 2 inocula of IMRD and Abbott were stored for 15 and 13 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 20, 1978. Seeds were covered with 1,400 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in late January 1979. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation eliminated the low populations of organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 41, 6, 2, and <1 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on 6 to 19 percent of short roots on control seedlings; no fruit bodies were observed at that time.

At termination of the study, seedlings that received either IMRD or Abbott inocula did not produce Pt indices >50 (Table 7). Abbott inoculum significantly decreased various growth parameters of seedlings in comparison to control seedlings. Pt ectomycorrhizae from IMRD inoculum did not influence seedling growth. Seedling density varied from 194 to 462 seedlings/m<sup>2</sup> (average 320/m<sup>2</sup>) and was not affected by treatment. Fruit bodies of Pt were not observed. Naturally occurring ectomycorrhizae resembled those formed by Tt; its fruit bodies occurred on over 15 percent of the sampled seedlings.

*Waynesboro Nursery, Waynesboro, Mississippi.*—During fumigation in this state nursery, day air temperature (sunny to overcast) averaged 24°C and soil moisture was approximately 25 percent of field capacity with numerous clods up to 15 cm in diameter. Plastic covering was removed after 5 days. Batch 2 inocula of IMRD and Abbott were stored for 15 and 13 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 20, 1978. Seeds were covered with a 2.5-cm layer of nonfumigated pine straw. Midstudy root assessments were made in early August, and the study was terminated in December. Seedlings were considered culls if shorter than 15 cm or with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated the low populations of organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 88, 26, 11, and 7 for IMRD and the three decreasing rates of Abbott

inocula, respectively; a trace of *Pt* ectomycorrhizae (*Pt* index 1) was detected on control seedlings. Naturally occurring ectomycorrhizae occurred on 6 to 19 percent of short roots on control seedlings; no fruit bodies were observed at that time.

At termination of the study, seedlings that received IMRD inoculum had a *Pt* index of 57; Abbott inoculum was ineffective (Table 7). Based on the amount of naturally occurring *Pt* ectomycorrhizae on control seedlings, 5 percent of the *Pt* ectomycorrhizae on seedlings in the IMRD and Abbott inoculum plots was probably due to its natural occurrence. Treatments had little effect on seedling growth, but *Pt* ectomycorrhizae from IMRD inoculum significantly reduced seedling culls by over half in comparison to control seedlings. Seedling density averaged 215/m<sup>2</sup> and was not affected by treatment. *Pt* fruit bodies were not observed but fruit bodies of *Tt* occurred on 10 to 28 percent of sampled seedlings.

*Buckeye Cellulose Corporation Nursery, Florida.*—Soil conditions during fumigation were similar to those in the 1977 study. Batch 2 inocula of IMRD and Abbott were stored for 5 and 3 days, respectively, before use. Inoculum was applied and nontreated seeds of slash pine were planted on April 10, 1978. Seeds were covered with 1,120 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in January 1979. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed *Pt* indices of 62 and <1 for IMRD and Abbott inocula. A total of nine fruit bodies of *Pt* were observed at that time in the IMRD inoculum plots. More than eight fruit bodies of *R. nigrescens* and a few of *Tt* were also observed in each plot. Naturally occurring ectomycorrhizae, mainly a white, complex coralloid type, occurred on 25 to 36 percent of short roots on control seedlings. White hyphal strands were traced from ectomycorrhizae to fruit bodies of *R. nigrescens*.

At termination of the study, neither IMRD nor Abbott inocula produced a *Pt* index >50 on seedlings (Table 7). Treatments had no significant effect on seedling growth or number of culls. Seedling density averaged 273/m<sup>2</sup> and was not affected by treatment. Four additional *Pt* fruit bodies were detected in IMRD inoculum plots at the end of the study. *Pt* fruit bodies varied in diameter from 4 to 14 cm, and about half were stalked. The naturally occurring ectomycorrhizae resembled those formed by *R. nigrescens* and *Tt*, and they occurred in approximately equal mixture. More than 11 fruit bodies of *R. nigrescens* were produced per m<sup>2</sup> of nursery bed during the growing season in the test plots. Fruit bodies of *Tt* occurred on 9 to 17 percent of sampled seedlings.

*Beauregard Nursery, Louisiana.*—Soil conditions during fumigation were similar to those in the 1977 study. Batch 2 inocula of IMRD and Abbott were stored for 6 and 4 days, respectively, before use. Inoculum was applied and thiram-treated seeds of longleaf pine were planted on April 11, 1978. Seeds were covered with a 2-cm layer of nonfumigated pine straw. Midstudy root assessments were made in August, and the study was terminated in January 1979. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation did not eliminate all Pythiaceae fungi and nematodes (Table 6). Heavy rains occurring shortly after installation of the study washed seeds from plots and reduced seedling density. Midstudy assessments of ectomycorrhizae on the seedlings revealed *Pt* indices of 79, 9, 14, and 3 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on 5 to 21 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings that received IMRD inoculum had a *Pt* index of 83; Abbott inoculum was ineffective (Table 7). *Pt* ectomycorrhizae from

IMRD inoculum significantly increased seedling growth in comparison to control seedlings but did not reduce the high incidence of seedling culls. Seedling density averaged only 168/m<sup>2</sup> and was not affected by treatment. The only fruit bodies observed were those of Tt which occurred on 8 to 19 percent of sampled seedlings. Naturally occurring ectomycorrhizae resembled those formed by this fungus.

*Union State Nursery, Jonesboro, Illinois.*—During fumigation in this state nursery, neither soil nor air temperature was recorded but soil moisture was near field capacity with many clods. The plastic covering was removed after 3 days. Batch 4 inocula of IMRD and Abbott were stored for 8 and 6 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 26, 1978. Mulch was not used. Midstudy root assessments were made in August, and the study was terminated in early April 1979. Seedlings were considered culls if shorter than 20 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the loblolly pine seedlings revealed Pt indices of 40, 27, 21, and 12 for IMRD and the three decreasing rates of Abbott inocula, respectively. No fruit bodies were detected; naturally occurring ectomycorrhizae, which resembled those formed by Tt, occurred on 26 to 38 percent of short roots on control seedlings at that time.

At termination of the study, neither IMRD nor Abbott inoculum produced Pt indices >50 on seedlings (Table 7). Treatment had no effect on seedling growth or number of culls in comparison to control seedlings. Fruit bodies of ectomycorrhizal fungi were not detected. Seedling density averaged 383/m<sup>2</sup> and was not affected by treatment. All naturally occurring ectomycorrhizae resembled those formed by Tt.

*George O. White Nursery, Licking, Missouri.*—During fumigation in this state nursery, day air temperature (sunny) reached 30°C and soil moisture was approximately 40 percent of field capacity. Plastic covering was removed after 5 days. Batch 4 inocula of IMRD and Abbott were stored for 7 and 5 days, respectively, before use. Inoculum was applied and anthraquinone-treated seeds of shortleaf pine were planted on April 25, 1978. Seeds were covered with a 2-cm layer of oak-planer shavings as a mulch. Midstudy root assessments were made in August, and the study was terminated in April 1979. Seedlings were considered culls if shorter than 10 cm and with root-collar diameters less than 2 mm.

*Results:* Neither Pythiaceae fungi or pathogenic nematodes were detected in pre- or post-fumigation soil samples (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 33, 11, 44, and 13 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, occurred on only 5 to 10 percent of short roots on control seedlings; fruit bodies were not detected at that time.

At termination of the study, neither IMRD nor Abbott inocula produced a Pt index >50 on seedlings (Table 7). Treatment had no effect on seedling size, total degree of ectomycorrhizal development, or number of culls in comparison to control seedlings. There were 11, 1, and 1 Pt fruit bodies produced in plots of IMRD and the highest rates of Abbott inocula, respectively. They varied in size from 1 to 4 cm in diameter and all were sessile. Seedling density averaged 760/m<sup>2</sup> and was not affected by treatment. This high seedling density may have negated inoculum treatment effects.

*Vallonia State Nursery, Vallonia, Indiana.*—During fumigation in this nursery, day air temperature (overcast) reached 15°C and soil moisture was 75 percent of field capacity. Plastic covering was removed after 3 days. Batch 4 inocula of IMRD

and Abbott were stored for 30 and 28 days, respectively, before use. Inoculum was applied and thiram-treated seeds of Virginia pine were planted on May 18, 1978. Seeds were covered with 2,240 kg/ha of hydromulch. Midstudy root assessments were made in August, and the study was terminated in March 1979. Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 3 mm.

**Results:** Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 27, 16, 0, and 1 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, occurred on 5 to 9 percent of short roots; no fruit bodies were detected at that time.

At termination of the study, seedlings that received IMRD and the highest rate of Abbott inocula had Pt indices over 80; other Abbott inoculum rates averaged 22 (Table 7). Treatments had no significant effect on seedling size or number of culls in comparison to control seedlings. Totals of 28, 88, 0, and 5 Pt fruit bodies were produced in plots of IMRD and the three decreasing rates of Abbott inocula, respectively. They varied from 0.5 to 9 cm in diameter and all were sessile. Tt fruit bodies occurred on 6 percent of test seedlings. Seedling density averaged 155/m<sup>2</sup> and was not affected by treatment.

*Tyee Tree Farm Nursery, Umpqua, Oregon.*—During fumigation in this new private nursery, day air temperature (overcast with rain) reached 12°C and soil moisture was approximately 80 percent of field capacity. Plastic covering was removed after 2 months. Batch 4 inocula of IMRD and Abbott were stored for 21 and 19 days, respectively, before use. Inoculum was applied and nontreated seeds of the coastal variety of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were planted on May 18, 1978. Mulch was not used. Midstudy root assessments were made in November, and the study was terminated in November 1979. Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 3 mm.

**Results:** Soil fumigation eliminated all but a few Pythiaceae fungi (Table 6). Post-fumigation soil samples were assayed in December 1977 and again in May 1978; there were no significant changes in populations of organisms assayed between the two sampling periods. Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 7, 3, 12, and 6 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae occurred on only 4 to 6 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, only a trace of Pt ectomycorrhizae was observed on seedlings from IMRD inoculum; none were detected on seedlings from Abbott inoculum (Table 7). Inoculum treatments did not significantly affect seedling growth but Abbott inoculum at 0.54 l/m<sup>2</sup> significantly decreased total ectomycorrhizal development on seedlings. Seedling density and number of culls were not recorded. No fruit bodies of Pt were observed; Tt fruit bodies occurred on about 3 percent of the sampled seedlings.

*Coeur d'Alene Nursery, Coeur d'Alene, Idaho.*—During fumigation in this USDA Forest Service nursery, day air temperature (sunny) reached 25°C and soil moisture was approximately 50 percent of field capacity. The plastic covering was removed after 6 days. Batch 4 inocula of IMRD and Abbott were stored for 17 and 15 days, respectively, before use. Inoculum was applied and nontreated seeds of the inland variety of Douglas-fir were planted on May 4, 1978. A 1-cm layer of nontreated sand was used as a mulch. Midstudy root assessments were made in October, 1978, roots were pruned laterally in July 1979, and the study was ter-

minated in November 1979. Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 3 mm.

*Results:* No assayed organisms were detected in soil samples collected after fumigation (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 2, 1, 2, and 0 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae were detected on only 3 to 5 percent of short roots on control seedlings; no fruit bodies were observed at that time. Lateral roots supporting short roots on the seedlings were sparse and discolored in all treatments.

At termination of the study, Pt ectomycorrhizae were not observed on any seedlings (Table 7). Inoculum treatments did not affect seedling growth. Seedling density and number of culls were not recorded. Fruit bodies of Pt were not observed; Tt formed fruit bodies on 2 percent of the sampled seedlings. The naturally occurring ectomycorrhizae were a distinctive white, complex coralloid type that strongly resembled Pt ectomycorrhizae in morphology.

*Parsons State Nursery, Parsons, West Virginia.*—During fumigation in this nursery, soil temperature at 15 cm depth reached 18°C and soil moisture was approximately 20 percent of field capacity. Plastic covering was removed after 3 days. Batch 4 inocula of IMRD and Abbott were stored for 10 and 8 days, respectively, before use. Inoculum was applied and thiram-treated seeds of eastern white pine (*P. strobus* L.) were planted on April 28, 1978. A 3-cm layer of rye grass straw was placed over seeds as a mulch. Midstudy root assessments were made in October, and the study was terminated in March 1980. Seedlings were considered culls if shorter than 10 cm and with root-collar diameters less than 3 mm.

*Results:* No organisms assayed were detected in soil samples collected in December after fall 1977 fumigation. However, low populations were detected in soil resampled in April 1978 before installation of the study (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 66 and <1 for IMRD and the Abbott inoculum treatments, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 24 to 42 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 47; Abbott inoculum was ineffective (Table 7). Pt ectomycorrhizae did not affect seedling growth or number of culls but significantly increased total ectomycorrhizal development in comparison to control seedlings. Seedling density varied from 84 to 919 seedlings/m<sup>2</sup> (average 450/m<sup>2</sup>) and was not affected by treatments. Variation in seedling density caused variation in seedling growth which may have precluded treatment effects. Moderate to severe necrosis of feeder roots was observed on certain seedlings but it did not appear to be related to treatment. No fruit bodies of Pt were detected; Tt formed fruit bodies on 11 percent of sampled seedlings.

*Marietta State Nursery, Marietta, Ohio.*—During fumigation in this nursery, soil temperatures were not recorded and soil moisture was approximately 50 percent of field capacity. Plastic covering was removed after 3 days. Batch 4 inocula of IMRD and Abbott were stored for 25 and 23 days, respectively, before use. Inoculum was applied and thiram-treated seeds of eastern white pine were planted on May 12, 1978. Seeds were covered with a 1-cm layer of sawdust and 1,680 kg/ha of hydromulch. Midstudy root assessments were made in October, and the study was terminated in April 1980. Seedlings were considered culls if shorter than 15 cm and with root-collar diameters less than 4 mm.

*Results:* No organisms assayed were detected in soil samples collected in October after fall 1977 fumigation but low populations were found in samples collected in May 1978 before installation of the study (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 37 and <1 for IMRD and Abbott inocula, respectively. Pt ectomycorrhizal development was highly variable among blocks of the same treatment. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 4 to 19 percent of short roots on control seedlings at that time.

At termination of the study, neither inoculum source formed a Pt index >50 on seedlings (Table 7). Certain rates of Abbott inoculum inhibited various parameters of seedling growth and all inoculum sources and rates significantly reduced seedling height in comparison to control seedlings. Seedling density varied from 253 to 451 seedlings/m<sup>2</sup> (average 335/m<sup>2</sup>) which may have precluded treatment effects. Fruit bodies of ectomycorrhizal fungi were not observed during the 2 years of this study.

*Oklahoma State Nursery, Washington, Oklahoma.*—During fumigation in this nursery, soil temperature at 15 cm depth reached 22°C and soil moisture was approximately 50 percent of field capacity. Plastic covering was removed after 4 days. Batch 4 inocula of IMRD and Abbott were stored for 22 and 20 days, respectively, before use. Inoculum was applied and thiram-treated seeds of Austrian pine (*P. nigra* Arnold) were planted on May 9, 1978. Seeds were covered with a 1-cm layer of partially decayed sawdust as a mulch. Midstudy root assessments were made in December 1978, and the study was terminated in December 1979. Seedlings were considered culls if shorter than 10 cm and with root-collar diameters less than 5 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed indices of 88, 61, 79, and 73 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 5 to 11 percent of short roots on control seedlings; no fruit bodies were observed at that time.

At termination of the study, the Pt indices on seedlings dropped dramatically to 14, 4, 5, and 9 for IMRD and the three decreasing rates of Abbott inocula, respectively (Table 7). Pt ectomycorrhizae formed by IMRD and the two highest rates of Abbott inocula significantly increased seedling growth, and all inoculum treatments significantly decreased culls by nearly half in comparison to control seedlings. Seedling density was highly variable (16 to 222 seedlings/m<sup>2</sup>). The highest rate of Abbott inoculum had an average seedling density of only 92 seedlings/m<sup>2</sup>; other inoculum treatments had significantly more seedlings (average 137/m<sup>2</sup>). Totals of 2, 1, 3, and 5 fruit bodies of Pt were found in plots of IMRD and the three decreasing rates of Abbott inocula, respectively. All were stalked and varied from 3 to 6 cm in diameter. Fruit bodies of other ectomycorrhizal fungi were not detected.

*New Kent Nursery, Virginia.*—Soil temperature averaged 14°C and the weather was overcast to rainy during fumigation. Batch 5 inocula of IMRD and Abbott were stored for 10 and 8 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on May 5, 1978. Mulch was not used. Midstudy root assessments were made in early August, and the study was terminated in February 1979. Characteristics of cull seedlings were the same as 1977.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 61, 24, 40, and 17 for IMRD and the three decreasing rates of Abbott inocula, respectively; a few Pt ectomycorrhizae (Pt index = 0.2) were found on control seedlings. Nat-

urally occurring ectomycorrhizae occurred on 21 to 40 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings that received IMRD inoculum had a Pt index of 86; Abbott inoculum produced very low indices (Table 7). Pt ectomycorrhizae from IMRD inoculum did not affect seedling size or number of culls in comparison to control seedlings. Abbott inoculum significantly decreased top weight ( $0.54 \text{ l/m}^2$ ) and total ectomycorrhizal development ( $1.08 \text{ l/m}^2$ ). Totals of 11, 2, and 1 Pt fruit bodies were observed in plots of IMRD and the two highest rates of Abbott inocula, respectively. These varied in size from 1 to 8 cm in diameter; about half were stalked. Tt fruit bodies occurred on 18 percent of test seedlings. Seedling density averaged  $561/\text{m}^2$  and was not affected by treatment.

*Industrial Forestry Association Nursery, Toledo, Washington.*—During fumigation in this forest industry cooperative nursery, day air temperature (sunny) reached  $15^\circ\text{C}$  and soil moisture was at field capacity. Plastic covering was removed after 7 days. Batch 5 inocula of IMRD and Abbott were stored for 31 and 29 days, respectively, before use. Inoculum was applied and nontreated seeds of the coastal variety of Douglas-fir were planted on May 18, 1978. Mulch was not used to cover seeds. Records of fertilizers and pesticides added during both growing seasons were not available. Midstudy root assessments were made in October 1978, and the study was terminated in October 1979. Seedlings were considered culls if shorter than 20 cm and with root-collar diameters less than 2 mm.

*Results:* Low levels of organisms assayed were detected in soil samples collected in the spring after fumigation in the fall of 1977 (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed a Pt index of 2 and 0 for IMRD and Abbott inoculum. Naturally occurring ectomycorrhizae occurred on 6 to 10 percent of short roots on control seedlings; Tt fruit bodies occurred on 4 percent of seedlings at that time.

At termination of the study, Pt ectomycorrhizae were not detected on any seedlings (Table 7). Unfortunately, sample seedlings were in transit 8 days between the nursery and the IMRD, and they arrived in a moldy condition. Assessments of ectomycorrhizae were made difficult by this condition. Seedling growth was not affected by treatments in comparison to control seedlings. Seedling density and number of culls were not determined. Naturally occurring ectomycorrhizae, mainly a white pinnate to complex coralloid type, were apparently formed by Tt and *Laccaria laccata* (Scop. ex Fr.) Cooke. Fruit bodies of both fungi were observed in most plots; Tt formed fruit bodies on nearly a third of all sampled seedlings. Pt fruit bodies were not observed.

*Bessey Tree Nursery, Nebraska.*—Soil conditions during fumigation were similar to those in 1977. Batch 5 inocula of IMRD and Abbott were stored for 16 and 14 days, respectively, before use. Inoculum was applied and nontreated seeds of Austrian pine were planted on May 12, 1978. Mulch was not used. Roots were laterally pruned in June and September 1979 and vertically pruned in October 1979. Midstudy root assessments and characteristics of cull seedlings were the same as in 1977. The study was terminated in March 1980.

*Results:* Soil fumigation eliminated all but a few saprophytic nematodes (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 63, 22, 13, and 4 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, primarily a white, complex coralloid type, were found on 7 to 21 percent of short roots on control seedlings; no fruit bodies were found at that time.

At termination of the study, no inoculum treatment produced a Pt index  $>50$ . Seedlings in the IMRD inoculum treatment had larger stem diameters and a greater Pt index than those of other treatments. Other growth or plot parameters were not affected. Seedling density averaged  $292/\text{m}^2$ . No fruit bodies of Pt or other

fungi were observed. Naturally occurring ectomycorrhizae appeared to be formed equally by Tt and the fungus forming the aforementioned white pinnate to complex coralloid type.

*Edwards State Nursery, Morganton, North Carolina.*—During fumigation in this nursery, day air temperature (sunny) reached 24°C and soil moisture was approximately 50 percent of field capacity. The plastic covering was removed after 2 days. Batch 5 inocula of IMRD and Abbott were stored for 23 and 21 days, respectively, before use. Inoculum was applied and thiram-treated seeds of eastern white pine were planted on May 18, 1978. Seeds were covered with a 1-cm layer of wheat straw as a mulch. Midstudy root assessments were made in November 1978, and the study was terminated in December 1979. Seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 2 mm.

*Results:* Low populations of Pythiaceae fungi were found in soil samples collected in May 1978 after fall 1977 fumigation (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 50, 5, <1, and <1 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 11 to 33 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings from either inoculum source had Pt indices >50, but IMRD and the highest rate of Abbott inoculum consistently produced Pt ectomycorrhizae (Pt indices of 48 and 35, respectively) that were intermixed with abundant naturally occurring ectomycorrhizae (Table 7). All ectomycorrhizae were large, nodular types with as many as 100, 2- to 6-mm long, dichotomous tips per ectomycorrhiza. Pt ectomycorrhizae formed by IMRD and the highest rate of Abbott inocula significantly increased seedling growth in comparison to control seedlings. These treatments also reduced culls from 41 percent in the control seedlings to 19 and 27 percent, respectively. Seedling density averaged 219/m<sup>2</sup> and was not affected by treatment. Totals of 13, 2, and 1 Pt fruit bodies were formed by IMRD and the two highest rates of Abbott inocula, respectively. They varied from 3 to 7 cm in diameters; three were sessile. Fruit bodies of other ectomycorrhizal fungi were not detected, however, naturally occurring ectomycorrhizae resembled those formed by Tt.

*Potlatch Corporation Nursery, Cloquet, Minnesota.*—During fumigation in this industrial nursery, soil temperature (sunny) reached 21°C and soil moisture was not monitored. The plastic covering was removed after 3 days. Batch 5 inocula of IMRD and Abbott were stored for 47 and 45 days, respectively, before use. Inoculum was applied and captan-treated seeds of red pine (*P. resinosa* Ait.) were planted on June 13, 1978. Mulch was not used. Midstudy root assessments were made in October 1978 and October 1979, and the study was terminated in May 1980. Seedlings were considered culls if shorter than 4 cm and with root-collar diameters less than 2 mm.

*Results:* Soil fumigation eliminated most of the organisms assayed (Table 6). The first assessment of ectomycorrhizae in October 1978 on the seedlings revealed Pt indices of 61, 12, 19, and 1 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on only 1 to 5 percent of short roots on control seedlings; no fruit bodies were detected at this time. In October 1979, the second root assessment revealed Pt indices of 81, 2, 2, and 5 for the respective inoculum treatments. Tt fruit bodies were observed on 3 percent of sampled seedlings; between 20 and 44 percent of short roots on control seedlings were ectomycorrhizal at that time.

At termination of the study, neither inoculum source had produced a Pt index

>50 although IMRD inoculum consistently produced *Pt* ectomycorrhizae (*Pt* index 42) on the majority of seedlings (Table 7). *Pt* ectomycorrhizae from IMRD and the two highest rates of Abbott inocula significantly increased seedling growth in comparison to control seedlings. Seedling density was very high, averaging 586/m<sup>2</sup> and was not affected by treatment. Seven *Pt* fruit bodies were observed in IMRD inoculum plots and *Tt* formed fruit bodies on more than 5 percent of sampled seedlings.

*Nepco Lake Nekoosa-Edwards Corporation Nursery, Port Edwards, Wisconsin.*—During fumigation in this forest industry nursery, soil temperature at 15 cm depth reached 10°C and soil moisture was not monitored. Plastic covering was removed after 12 days. Batch 5 inocula of IMRD and Abbott were stored for 16 and 14 days, respectively, before use. Inoculum was applied and nontreated seeds of red pine were planted on May 11, 1978. Mulch was not used. Midstudy root assessments were made in October 1978 and October 1979; the study was terminated in April 1980. Seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 6). The first assessment of ectomycorrhizae in October 1978 on the seedlings revealed *Pt* indices of 64, 39, 27, and 12 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, mostly pinnate forms, were found on 10 to 25 percent of short roots on control seedlings; no fruit bodies were detected at that time. The second root assessment in October 1979 revealed *Pt* indices of 41, 38, 10, and 12 for the respective inoculum treatments. Naturally occurring ectomycorrhizae, which resembled those formed by *Tt*, occurred on 22 to 26 percent of short roots on control seedlings; *Tt* fruit bodies occurred on 22 percent of sampled seedlings at that time.

By termination of the study, *Pt* indices on seedlings had decreased to 15, 12, 4, and 9 for IMRD and the Abbott inocula rates, respectively (Table 7). *Pt* ectomycorrhizae did not affect seedling growth, but all inoculum treatments significantly decreased culls in comparison to control seedlings. Seedling density averaged 496/m<sup>2</sup> and was not affected by treatment. *Pt* fruit bodies were not observed but *Tt* fruit bodies occurred on 14 percent of all sampled seedlings.

*USDA-SCS Nursery, Newaygo, Michigan.*—During fumigation in this nursery, soil temperature at 15 cm depth reached 15°C and soil moisture was not monitored. Plastic covering was removed after 5 days. Batch 5 inocula of IMRD and Abbott were stored for 23 and 21 days, respectively, before use. Inoculum was applied and seeds of red lead-treated red pine were planted on May 18, 1978. Seeds were covered with an 0.8-cm layer of partially decayed sawdust as a mulch. Midstudy root assessments were made in November 1978, all plots were undercut in April 1979, and the study was terminated in April 1980. Seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation in fall 1977 did not eliminate all organisms assayed just before study installation in May 1978 (Table 6). Midstudy assessments of ectomycorrhizae on the seedlings revealed *Pt* indices of 58, 12, 17, and 9 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by *Tt*, were observed on 20 to 41 percent of short roots on control seedlings; no fruit bodies were detected at that time.

Just prior to termination of the study, seedlings in three test blocks were mistakenly lifted by nursery personnel and used as operational seedlings. The remaining two blocks were lifted and evaluated; statistical comparisons were not made. *Pt* indices of 72, 10, 18, and 2 were detected on seedlings in IMRD and the three Abbott inocula treatments, respectively (Table 7). Seedling growth, num-

ber of culls, and seedling density (571/m<sup>2</sup>) did not appear to be affected by treatment. Naturally occurring ectomycorrhizae were abundant; Tt formed fruit bodies on 22 percent of sampled seedlings. Fruit bodies of Pt were not observed.

*J. W. Toumey Nursery, Watersmeet, Michigan.*—During fumigation in this USDA Forest Service nursery, day air temperature (sunny) reached 10°C and soil moisture was approximately 30 percent of field capacity. Plastic covering was removed after 10 days. Batch 5 inocula of IMRD and Abbott were stored for 30 and 28 days, respectively, before use. Inoculum was applied and thiram-treated seeds of red pine were planted on May 25, 1978. Seeds were covered with 784 kg/ha of hydromulch. Midstudy root assessments were made in October 1978 and November 1979, and the study was terminated in May 1981. Seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 4 mm.

*Results:* Soil fumigation eliminated all but a few saprophytic nematodes (Table 6). The first assessment of ectomycorrhizae on the seedlings in October 1978 revealed Pt indices of 79, 44, 27, and 9 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 5 to 11 percent of short roots on control seedlings; no fruit bodies were detected at that time. The second root assessment made in November 1979 showed that Pt indices had decreased to 5, 3, 2, and 3 for the respective inocula treatments. Naturally occurring ectomycorrhizae occurred on an average of 44 percent of short roots.

When the study ended, only a trace of Pt ectomycorrhizae was detected on seedlings regardless of inoculum treatment (Table 7). Seedling growth was not significantly affected by treatment, but number of culls was significantly reduced by IMRD and the lowest rate of Abbott inocula in comparison to control seedlings. Seedling density averaged 152/m<sup>2</sup> and was not affected by treatment. Naturally occurring ectomycorrhizae were abundant; Tt formed fruit bodies on more than 50 percent of sampled seedlings and the jet-black ectomycorrhizae formed by *Cenococcum geophilum* Fr. (syn. *C. graniforme*) were also observed on 18 percent of the seedlings.

#### DISCUSSION

The three batches of IMRD inoculum produced excellent Pt indices on loblolly pine in the IMRD Microplot Nursery tests, but only batch 5 of Abbott inoculum produced a Pt index >50 (Table 7). All of these inocula produced Pt fruit bodies as well. Abbott batches 2 and 4 produced only a few Pt ectomycorrhizae and no fruit bodies.

Moisture content apparently had no effect on inoculum efficacy. The relatively dry (7.6 percent moisture content) IMRD batch 4 was as effective as batch 5, which contained nearly 2.5 times more moisture (18.2 percent moisture content). All batches of Abbott inoculum were consistent in regard to bulk density and moisture content (Table 5).

In the conventional nursery 1-0 pine tests, Abbott inoculum batch 2 not only failed to produce Pt indices >50 but also formed very few Pt ectomycorrhizae. Also, only one fruit body was produced by this inoculum in eight nursery tests. This inoculum caused a decrease in seedling growth in two nurseries and significantly increased number of culls in two others.

In the eight nursery tests involving Abbott inoculum batch 4, only one at the highest rate on Virginia pine in the Vallonia State Nursery produced a Pt index >50. Seedling growth and number of culls were not significantly affected by this treatment. In this treatment, a large number of small (1.0 to 1.5 cm in diameter) Pt fruiting bodies were produced. This inoculum significantly increased seedling growth and significantly decreased culls in the Oklahoma State Nursery and significantly decreased seedling growth in the Marietta State Nursery. However, in both nurseries it is unlikely that the differences were related to Pt ectomycorrhizal

development because the percentage of roots colonized was very low. Differences may have been due to variable seedbed densities. It should also be pointed out that IMRD inoculum produced final Pt indices < 50 in seven of the eight nurseries in which this batch of Abbott inoculum was tested. However, IMRD inoculum produced a Pt index of 66 on eastern white pine in the Parsons State Nursery after the first year even though 1,025 kg of N/ha (Appendix VI) was applied during the growing season. When inoculum was applied the nursery soil also contained a high amount of total N (1,184  $\mu\text{g/g}$ ). Apparently high rates of N are not as inhibitory to Pt ectomycorrhizal development as suspected.

In the eight nursery tests involving Abbott inoculum batch 5, no acceptable Pt indices were obtained. However, compared with other Abbott batches, batch 5 produced consistently more Pt ectomycorrhizae and fruit bodies. In four of these nurseries, IMRD inoculum was no more effective on seedlings than this batch of Abbott inoculum. IMRD inoculum produced Pt indices > 50 in only two of these eight nurseries. This Abbott inoculum significantly increased seedling growth in two nurseries and significantly decreased culls in three nurseries; these differences appeared to be correlated with high midstudy Pt indices.

In the 12 conventional nursery 1-0 pine tests, IMRD inoculum produced acceptable Pt indices in eight nurseries. Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth in five nurseries, significantly decreased culls in three nurseries, and significantly increased total ectomycorrhizal development in six nurseries. Only in one nursery without an acceptable Pt index did IMRD inoculum significantly affect seedlings and that was on slash pine at the Buckeye Cellulose Corporation Nursery, where total ectomycorrhizal development was significantly increased.

One feature of all batches of 1977 and 1978 Abbott inoculum different from the more effective IMRD inoculum was that the Abbott inocula were not leached. High amounts of carbohydrates and other nutrients in the nonleached inoculum could have served as energy sources for saprophytic microorganisms in the nursery soil. Microbial colonization of the nutrient-rich particles of Abbott inoculum could have been antagonistic to Pt mycelium in the particles, thereby causing a loss of survival and effectiveness in forming ectomycorrhizae. The importance of leaching inoculum has been discussed (Marx 1980).

The value of the midstudy assessments of the 1-0 pine seedling tests was verified. Overall, midstudy Pt indices for IMRD inoculum averaged 62 compared with final Pt indices of 66. Midstudy Pt indices in six nurseries were higher, five were lower, and one remained the same compared with final assessments in these 12 nurseries. Although the low Pt index obtained on loblolly pine in 1977 in the Weyerhaeuser Nursery, Oklahoma, was improved in the 1978 tests, the Pt index at the Weyerhaeuser Nursery, Arkansas, was still unacceptable.

In the conventional nursery 2-0 pine and Douglas-fir tests (excluding the USDA-SCS Nursery), IMRD inoculum did not produce a Pt index > 50. A Pt index of 72 was obtained on red pine with IMRD inoculum in the USDA-SCS Nursery but, unfortunately, this index was based on only two of five replicate blocks. Even though Pt indices > 50 were not obtained on seedlings in any of these ten 2-0 nurseries, Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth and significantly reduced culls in three nurseries, and significantly increased total ectomycorrhizal development in six nurseries.

The significant increase in the growth of 2-0 red pine seedlings by IMRD and Abbott inocula at the Potlatch Corporation Nursery has important implications for production of red pine seedlings in north-central United States. Plantable-size 2-0 red pine seedlings are highly desirable and economically advantageous compared with the conventional 3-0 seedling rotations.

In several nurseries, Pt index varied from block to block within the same treatment and acceptable and unacceptable Pt indices occurred. For example, on loblolly pine in Union State Nursery, IMRD inoculum produced Pt indices of

21, 14, 86, 76, and 3 in five blocks of seedlings for an average Pt index of only 40. However, seedlings in two of the five blocks had an average Pt index of 81 which is excellent. This variation indicates that the IMRD inoculum was potentially highly effective on the seedlings but that soil conditions or nursery cultural practices in certain test blocks limited its ability to form ectomycorrhizae.

Midstudy seedling assessments indicated that the IMRD inoculum produced Pt indices averaging 69 in 6 of the ten 2-0 seedling tests (excluding USDA-SCS Nursery). However, the average Pt index in these six nurseries decreased to 33 by the end of the second growing season. Midstudy Pt indices in all the 10 nurseries averaged 46 but final Pt indices averaged only 21.

Effectiveness of IMRD inoculum was very poor at the end of the studies in the three tests with 2-0 Douglas-fir and in the one test with 3-0 red pine. Based on midstudy Pt indices of 7, 2, and 2, respectively, the results with Douglas-fir were predictably unacceptable. However, in the 3-0 red pine test, root assessments at the end of the first growing season revealed high Pt indices for both IMRD and Abbott inocula. However, by the end of the second growing season, Pt indices of 5 or lower indicated a rapid supplantation of Pt ectomycorrhizae. A decrease in or supplantation of Pt ectomycorrhizae formed on seedlings during the first growing season in the 2-0 and 3-0 tests may have been due to winter kill or natural mortality of Pt ectomycorrhizae and subsequent formation of ectomycorrhizae by naturally occurring fungi on new roots formed at the beginning of the new growing season. Apparently Pt was not able to spread as rapidly to newly formed roots in the spring as could the other, perhaps more cold-adapted, nursery fungi during the second and third growing seasons.

When the various procedures and cultural practices used by nurseries in the 1-0 pine tests with IMRD inoculum were compared, no one cultural practice accounted for the differences in Pt index. The four nurseries with Pt indices <50 were properly fumigated, and had inoculum storage times, soil fertility levels, cover crops, and many pesticide applications similar to the eight nurseries with Pt indices >50. Two nurseries, Great Southern Paper Company and Beauregard, had bioassay indications of poor soil fumigation but had two of the highest Pt indices (79 and 85) observed in the 1978 tests. Although the 1977 tests at the IMRD Microplot Nursery showed the importance of proper fumigation to effectiveness of inoculum, unknown factors not accounted for in our assay of Pythiaceus fungi and nematodes may also significantly affect inoculum effectiveness.

Soil fumigation in the fall was less effective than in the spring for successful inoculations. Neither of the two 1-0 pine tests and only three of the eight 2-0 and 3-0 pine and Douglas-fir tests that were fumigated in the fall had acceptable midstudy Pt indices from IMRD inoculum. However, 8 of the ten 1-0 pine tests and all 4 of the 2-0 pine tests which were fumigated in the spring had acceptable midstudy Pt indices. Fumigation in the fall may eliminate weed seed and most microorganisms including indigenous ectomycorrhizal fungi, but when the plastic covering is removed, usually in 2 to 5 days, the soil is exposed for several months to reinfestation by airborne saprophytes, pathogens, and ectomycorrhizal fungi. Basidiospores of Tt and other ectomycorrhizal fungi disseminated in air currents from fruit bodies in adjacent forest or areas of the nursery still growing pine seedlings could effectively inoculate the fumigated soil. This colonization, as well as buildup of potentially antagonistic microflora, could preclude effectiveness of Pt inoculum.

Results of 12 tests with container-grown seedlings in six widespread forest research locations in North America (Marx and others 1982) showed that a separate batch of Abbott inoculum produced in 1978 with identical procedures as the inoculum reported herein was effective in forming Pt indices >50 in five tests on pine, and one test each on bur oak (*Quercus macrocarpa* Michx.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Douglas-fir. In certain tests, the

Abbott inoculum was as effective as IMRD inoculum. Such results indicate that various cultural practices influence effectiveness of inoculum as much or more than do the different tree hosts.

Although the 1978 Abbott inoculum reported here was more effective than that produced in 1977, probably due to the addition of peat moss, it was still not as consistently effective as IMRD inoculum in nurseries. All 1978 (and 1977) batches of Abbott inoculum contained abundant mycelium of *Pt* inside and outside of the vermiculite particles, but these inocula may have contained sufficient quantities of unleached nutrients to limit effectiveness of the inoculum, inhibit seedling growth, and increase seedling culls as was observed in certain bare-root nursery tests. The significance of microbial contamination of inoculum during its production in fermentors or during leaching and drying was another factor needing study. Earlier work at the IMRD with jar cultures of *Pt* that were contaminated during incubation showed them to be relatively ineffective in forming *Pt* ectomycorrhizae. Further modifications in fermentation procedures and quality control at Abbott Laboratories appeared necessary to produce consistently effective batches of inoculum.

#### 1979 TESTS

Since lack of consistent quality of Abbott inoculum was identified as a problem, attempts were made to develop methods for rapidly assessing the effectiveness of inoculum batches before installation of elaborate and time-consuming nursery studies. The fastest method had been to test inoculum in containers using loblolly pine (Marx and others 1982). However, these tests required far too much time—a minimum of 12 to 14 weeks. Other assays were developed.

#### MATERIALS AND METHODS: INOCULUM CHARACTERIZATION

One assay involved determination of propagules of *Pt* in the inoculum particles. This was done by spreading 1 cc of dried inoculum (final product form) in a petri dish containing 10 ml of MMN agar medium fortified with 25 mg/l of benomyl (50 percent WP) and 10 mg/l erythromycin phosphate. These additives inhibited growth of contaminating fungi and bacteria. After the plates (10 per inoculum batch) were incubated at 30°C for 5 days, growth centers of the yellow-gold mycelium of *Pt* were counted. These centers were considered to represent individual propagules of *Pt* and were recorded as propagules per g of inoculum.

Microbial contamination was determined by blending 1 g of the final form of inoculum with 100 ml of sterile water for 3 minutes, serially diluting this blend by factors of 10, and placing 1 ml onto the surface of Difco trypticase soy agar. Plates (5 per dilution) were incubated for 5 days at 30°C and bacterial and fungal colonies were counted. The dilution yielding less than 10 colonies of bacteria or fungi per plate was considered the contamination level. To determine pH, inoculum was mixed thoroughly with distilled water in a 1:1 volume ratio and, after 30 minutes, pH was measured with a glass electrode. Residual glucose (mg glucose/g of oven-dried inoculum) in the inoculum was determined with an autoanalyzer by a modification of Hoffman's (1937) technique.

IMRD inoculum was produced and processed, as discussed earlier, then shipped to Abbott Laboratories for all the above characterizations except for determination of bulk density, pH, and moisture content. Due to shipment time between Athens, Georgia, and Chicago, Illinois, several days elapsed between characterization of IMRD and the Abbott inoculum; the latter inoculum was characterized immediately after drying at the Abbott facility.

The 1979 Abbott inoculum was produced in rotary-drum fermentors containing vermiculite and nutrients. The substrate was steamed, cooled, and inoculated with

TABLE 8. Characteristics of vegetative inoculum of *Pisolithus tinctorius* (Pt) produced by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories in 1979.<sup>1</sup>

Inoculum	Bulk density (g/l)	Moisture content (percent)	Propa- gules Pt (Pt/g)	Residual glucose (mg/g)	pH	Contaminants	
						Bacteria dilution	Fungi dilution
IMRD							
Batch 1	320	6.4	1	4.7	5.0	$8.0 \times 10^6$	$6.9 \times 10^6$
Batch 2 <sup>2</sup>	363	53.1	—	—	4.7	—	—
Batch 3	297	27.4	5	6.7	4.6	$2.9 \times 10^7$	$3.5 \times 10^6$
Batch 4	325	51.4	7	4.6	4.6	$3.0 \times 10^7$	$1.0 \times 10^6$
Abbott							
Batch 1	266	22.0	38	—	6.0	$<10^2$	$5.0 \times 10^2$
Batch 2	238	22.6	19	1.4	8.0	$6.7 \times 10^7$	$<10^3$
Batch 3	265	21.0	18	6.5	6.4	$<10^2$	$<10^2$
Batch 4	220	21.0	32	—	7.2	$<10^3$	$<10^3$

<sup>1</sup> Details of methods used to obtain characteristics are presented in text.

<sup>2</sup> Inoculum was lost in shipment from IMRD to Abbott Laboratories; therefore, characterizations were not made.

starter mycelium of Pt. Starter mycelium was grown as previously described. The culture was incubated in the fermentor, leached, removed, and dried. Since peat moss was difficult to sterilize it was excluded from the 1979 Abbott inoculum in an attempt to avoid microbial contamination.

Characterization of the different inocula showed that the different batches of IMRD inoculum had consistent bulk density, pH, and low residual glucose levels, but had highly variable moisture content and levels of microbial contaminants (Table 8). Very few propagules of Pt were isolated from vermiculite particles of IMRD inoculum. Batches of Abbott inoculum were consistent in moisture content, but variable in bulk density, pH, microbial contaminants, and residual glucose levels. Pt propagules/g of inoculum were much higher for each batch of Abbott inoculum than for any batch of IMRD inoculum. Both IMRD and Abbott inocula contained abundant hyphae of Pt inside and outside the vermiculite particles.

The 1979 conventional nursery tests involved four batches of IMRD and four batches of Abbott inoculum. The experimental designs and procedures used in the nurseries were identical to those of 1978. Because inoculum was stored for varying periods of time before use during the 1977 and 1978 tests, an inoculum storage study using the eight 1979 inoculum batches was installed at the IMRD Microplot Nursery to ascertain the significance of storage periods on inoculum effectiveness. Tests were installed also in four conventional nurseries per inoculum source and batch but 11 of the 16 tests were excluded from final evaluation due to various problems—very poor fumigation (detected after installation of study), soil and seed-washing rains, root-insect infestation, erratic seed germination, misuse of herbicides and organic amendments, and mislabeling of plots, as well as other problems detected during midstudy root assessment. Thus, only five nursery tests were carried to completion.

#### NURSERY INFORMATION AND RESULTS

Cropping history and cultural practices involved in the 1979 nursery tests prior to installation of the study are given in Appendix VII, chemical and physical

TABLE 9. Number of Pythiaceae fungal propagules (pg) and plant parasitic nematodes isolated from pre- and post-fumigation soil samples in nursery tests in 1979.

Nursery	Pythiaceae fungi <sup>1</sup> pg/g		Nematodes/475 cc soil	
	Pre-fum	Post-fum	Pre-fum	Post-fum
IMRD, GA	26	0	127 <sup>2,3,4</sup>	0
Buckeye, FL	37	0	29 <sup>2,5</sup>	0
International, MS	—	3	—	0 <sup>2</sup>
Champion, SC	—	1	—	0 <sup>2</sup>
Hiwassee, GA	23	0	81 <sup>2,3</sup>	0
Vallonia, IN	0	0	31 <sup>2,5,6</sup>	0

<sup>1</sup> Mainly *Pythium irregulare*.

<sup>2</sup> Saprophytic nematodes.

<sup>3</sup> Stunt nematode (*Tylenchorhynchus*).

<sup>4</sup> Ring nematode (*Criconeoides*).

<sup>5</sup> Dagger nematode (*Xiphinema*).

<sup>6</sup> Lesion nematode (*Pratylenchus*).

characteristics of soil at installation of the study are given in Appendix VIII, and cultural practices involved after installation of the study are given in Appendix IX; other details are as follows:

*IMRD Microplot Nursery, Georgia.*—Procedures and materials used were the same as those reported for the 1978 tests. Each of the four batches of Abbott inoculum, upon arrival at the IMRD, was packaged into 15 volumes of 400 ml each. Five volumes per batch were used immediately to infest soil in five microplots. The remaining inoculum was stored at 5°C, five volumes for 2 weeks and five volumes for 4 weeks, then used to inoculate soil as above. Batches of IMRD inoculum were processed, stored, and tested in an identical fashion. The experimental design was two sources of inoculum × four batches of each inoculum source × three inoculum storage periods × five replicate microplots in a randomized block design. Inoculum rate was 1.08 l/m<sup>2</sup> of soil surface. Since the various batches of inoculum were produced at different times, the tests were installed in the 120 microplots over a period of several weeks (April 9 through May 29, 1979). Midstudy root assessments of the loblolly pine seedlings were made in early August 1979 on all treatments; seedling ages, therefore, ranged from 71 to 122 days. Seedlings were lifted in December 1979 and assessed only for ectomycorrhizal development.

*Results:* Soil fumigation eliminated all organisms assayed (Table 9). Midstudy root assessments of the seedlings revealed that all batches of IMRD inoculum produced acceptable Pt indices after 0 and 2 weeks storage and that IMRD batches 1 and 3 were still highly effective after 4 weeks in storage (Table 10). At termination of the study, only IMRD batch 2 with 4 weeks of storage did not produce Pt index >50. Each batch of IMRD inoculum produced fruit bodies of Pt; these were all stalked and had average diameters of 4.2 cm.

Midstudy root assessments of seedlings with Abbott inoculum revealed that only batch 1 had a Pt index >50 when used immediately. However, after storage of 4 weeks it was less effective (Table 10). This loss of effectiveness attributed to storage was not evident at termination of the study where it was found that all storage treatments of Abbott batch 1 produced Pt indices >50. Two Pt fruit bodies (stalked, and 3 and 5 cm in diameter) were also produced in these plots. Other batches of Abbott inoculum, regardless of storage, were not effective.

TABLE 10. Development of ectomycorrhizae on loblolly pine seedlings by different batches of vegetative inoculum of *Pisolithus tinctorius* (Pt) produced in 1979 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and stored at 5°C for different periods.<sup>1</sup>

Inoculum source	Storage (weeks)	Midstudy assessment <sup>2</sup>				Final assessment <sup>2</sup>				Total number Pt fruiting bodies
		Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>3</sup>	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>3</sup>	
		Pt	All fungi			Pt	All fungi			
IMRD #1	0	42b	59ab	83ab	59bc	57b	67b	100a	85ab	3
	2	42b	63ab	92ab	61bc	54b	68b	100a	79b	0
	4	41b	62ab	79b	52bc	48bc	73ab	83b	55bc	1
IMRD #2	0	56ab	72ab	100a	78b	65ab	72ab	100a	90a	1
	2	33c	54b	92ab	56bc	45bc	66b	89b	61b	0
	4	17d	45b	67bc	25d	38c	57bc	71bc	47bc	0
IMRD #3	0	77a	83a	100a	93a	80a	91a	100a	88ab	3
	2	46b	59ab	89ab	69b	65ab	73ab	98a	87ab	0
	4	42b	60ab	83ab	58bc	48bc	65b	97a	72b	0
IMRD #4	0	82a	87a	100a	94a	81a	85a	100a	96a	1
	2	41b	63ab	93ab	61bc	77a	82a	100a	94a	1
	4	35c	62ab	74b	48cd	59b	69b	100a	86ab	0
Abbott #1	0	33c	59ab	97a	54bc	50b	59bc	100a	85ab	1
	2	31c	57b	94a	51c	71a	84a	100a	86ab	1
	4	26c	52b	71b	36d	50b	71ab	92a	65b	0
Abbott #2	0	14d	52b	38d	10e	17d	56bc	48c	15c	0
	2	0	48b	0	0	1e	50c	2d	<1d	0
	4	0	49b	0	0	0	53c	0	0	0
Abbott #3	0	2d	47b	26d	1e	11d	51c	38c	8d	0
	2	5d	44bc	18d	2e	16d	55bc	31c	9d	0
	4	0	48b	0	0	0	49c	0	0	0
Abbott #4	0	9d	52b	63bc	11e	18d	55bc	53c	17c	0
	2	2d	42c	17d	1e	8d	58bc	32c	4d	0
	4	0	48b	0	0	0	52c	0	0	0

<sup>1</sup> Numbers in the same column sharing a common letter are not significantly different at  $P = 0.05$ .

<sup>2</sup> Approximately 38 seedlings in each of 5 microplots were assessed at each period for each of the 24 treatments.

<sup>3</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

*Buckeye Cellulose Corporation Nursery, Florida.*—During soil fumigation, soil conditions were similar to those in 1977. Batch 1 inocula of IMRD and Abbott were stored for 8 and 13 days, respectively, before use. Inoculum was applied and nontreated seeds of slash pine were planted on April 7, 1979. Seeds were covered with 1,120 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in January 1980. Characteristics of cull seedlings were the same as in 1977.

*Results:* Soil fumigation eliminated all organisms assayed (Table 9). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 17, 47, 26, and 11 for IMRD and the three decreasing rates of Abbott inocula, respectively.

Naturally occurring ectomycorrhizae, mainly a white, complex coralloid type, were found on 20 to 44 percent of short roots on control seedlings; a few *R. nigrescens* fruit bodies were observed in all plots at that time.

At termination of the study, only the highest rate of Abbott inoculum produced Pt index > 50 on seedlings; IMRD inoculum did not (Table 11). Inoculum treatments had no effect on seedling size, total ectomycorrhizal development, or number of culls in comparison to control seedlings. Seedling density averaged 375/m<sup>2</sup> in inoculated plots; control plots had significantly more seedlings (419/m<sup>2</sup>). Totals of 4, 3, 5, and 4 Pt fruit bodies were observed in plots of IMRD and the three decreasing rates of Abbott inocula, respectively. Fruit bodies varied in size from 3 to 6 cm in diameter and about half were stalked. One stalked Pt fruit body from plots of Abbott inoculum was 17 cm tall. A total of 147 fruit bodies of *R. nigrescens* were found in the 25 test plots; an average of nearly 4/m<sup>2</sup> of soil surface. Only about 6 percent of the seedlings had fruit bodies of Tt on their stems. However, based on visual estimates of ectomycorrhizae, nearly half of all naturally occurring ectomycorrhizae were formed by this fungus; the remainder appeared to be formed by *R. nigrescens*.

*International Paper Company Nursery, Natchez, Mississippi.*—During fumigation in this new forest industry nursery, day air temperature (sunny) was 20°C and soil moisture was 50 to 60 percent of field capacity. Plastic covering was removed after 3 days. Batch 1 inocula of IMRD and Abbott were stored for 21 and 26 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 30, 1979. Seeds were covered with 1,568 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in December. Seedlings were considered culls if shorter than 18 cm and with root-collar diameters less than 4 mm.

*Results:* Soil fumigation did not eliminate all organisms assayed (Table 9). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 52, 16, 5, and 3 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae resembling those formed by Tt were found on 11 to 17 percent of short roots on control seedlings; one fruit body of Pt (4 cm in diameter and stalked) was produced in a plot of IMRD inoculum. Fruit bodies of other fungi were not detected at that time.

At termination of the study, seedlings that received IMRD and the highest rate of Abbott inocula had Pt indices of 74 and 69, respectively (Table 11). All inoculum treatments significantly increased seedling growth, but none significantly affected total ectomycorrhizal development or the number of cull seedlings in comparison to control seedlings. Seedling density averaged 442/m<sup>2</sup> and was not affected by treatment. Naturally occurring ectomycorrhizae resembled those formed by Tt; fruit bodies of this fungus occurred on 18 percent of sampled seedlings. A trace of Pt ectomycorrhizae was detected on control seedlings; this may be a natural occurrence from adjacent nursery sources or from spores released by Pt fruit bodies from nearby test plots prior to roguing.

*Champion-International Corporation Nursery, Swansea, South Carolina.*—During fumigation in this new forest industry nursery, day air temperature (sunny) was 20°C and soil moisture was approximately 40 percent of field capacity. Plastic covering was removed after 4 days. Batch 2 inocula of IMRD and Abbott were stored for 1 and 6 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 16, 1979. Seeds were covered with 1,120 kg/ha of hydromulch. Midstudy root assessments were made in early August, and the study was terminated in December. Seedlings were considered culls if shorter than 18 cm and with root-collar diameters less than 4 mm.

TABLE 11. Growth and ectomycorrhizal development of 1-0 pine seedlings from various nurseries with vegetative inocula of *Pisolithus tinctorius* (Pt) produced in 1979 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories.<sup>1</sup>

Location, species, inoculum source and rate (l/m <sup>2</sup> )	Height (cm)	Root collar diam- eter (mm)	Fresh weight (g)			Percent short roots ectomycor- rhizal with—		Per- cent seed- lings with Pt	Pt index <sup>2</sup>	Per- cent cull seed- lings
			Top	Root	Total	Pt	All fungi			
Buckeye, FL: slash pine										
IMRD 1.08	22.3a <sup>3</sup>	4.2a	10.1a	1.9a	12.0a	13a	27a	84a	42a	8a
Abbott #1, 2.16	23.0a	3.9a	8.7b	1.8a	10.5ab	16a	31a	94a	51a	10a
Abbott #1, 1.08	21.1a	3.8a	8.0b	1.5a	9.5b	7b	22a	88a	28b	11a
Abbott #1, 0.54	21.7a	4.1a	9.1ab	1.8a	10.9ab	7b	25a	82a	24b	11a
Control	21.7a	4.0a	9.2ab	1.8a	11.0ab	0	29a	0	0	10a
International, MS: loblolly pine										
IMRD 1.08	28.2a <sup>3</sup>	4.7a	10.4a	2.9a	13.3a	19a	26a	98a	74a	23a
Abbott #1, 2.16	29.7a	4.8a	10.9a	3.0a	13.9a	20a	29a	98a	69a	22a
Abbott #1, 1.08	27.2a	4.6ab	10.1a	2.3b	12.4a	10b	21a	70b	34b	24a
Abbott #1, 0.54	28.9a	4.8a	10.9a	2.8a	13.7a	9b	22a	58c	23b	16a
Control	22.4b	4.4b	7.8b	2.5ab	10.3b	3c	23a	22d	3c	29a
Champion, SC: loblolly pine										
IMRD 1.08	21.8a <sup>3</sup>	5.8a	11.5a	4.1a	15.6a	34a	50a	100a	69a	12a
Abbott #2, 2.16	20.7a	5.2b	9.2b	3.2b	12.4b	26b	46a	84b	51b	12a
Abbott #2, 1.08	22.7a	5.6ab	10.7ab	3.9ab	14.6ab	15c	47a	58c	19c	12a
Abbott #2, 0.54	21.0a	5.2b	9.0b	3.4b	12.4b	9c	46a	48c	9c	14a
Control	19.7a	5.3b	10.1ab	3.7ab	13.8b	0	47a	0	0	12a
Hiwassee, GA: loblolly pine										
IMRD 1.08	28.3a <sup>3</sup>	6.0a	14.8a	3.9a	18.7a	26a	47a	100a	57a	10a
Abbott #3, 2.16	28.2a	5.7ab	12.6ab	3.4ab	16.0ab	21a	37a	100a	59a	8a
Abbott #3, 1.08	28.1a	5.7ab	12.3b	3.4ab	15.7b	7c	33a	72b	17c	7a
Abbott #3, 0.54	29.3a	5.7ab	13.1ab	3.5ab	16.6ab	14b	42a	92ab	32b	9a
Control	27.4a	5.5b	11.7b	3.0b	14.7b	0	44a	0	0	9a
Vallonia, IN: Virginia pine										
IMRD 1.08	24.2a	3.9a	9.7ab	5.3b	16.0ab	38a	59a	100a	66a	40b
Abbott #4, 2.16	26.1a	4.2a	11.2a	5.9ab	17.1a	28b	53a	100a	54b	40b
Abbott #4, 1.08	25.1a	4.1a	11.8a	6.3a	18.1a	25b	51a	100a	51b	50a
Abbott #4, 0.54	22.9a	3.9a	8.8b	6.4a	15.2ab	23b	55a	70b	30c	48a
Control	25.1a	4.0a	9.8ab	5.1b	14.9b	1c	43b	10c	<1d	49a

<sup>1</sup> Means sharing a common letter in the same nursery but between inoculum treatments are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> Seedling tops mowed to approximately 20 cm at least once during growing season.

*Results:* Soil fumigation did not eliminate all organisms assayed (Table 9). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 51, 13, 11, and 3 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 18 to 32 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings that received IMRD and the highest rate of Abbott inocula had Pt indices of 69 and 51, respectively (Table 11). However, only Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth in comparison to control seedlings. None of the inoculum treatments had any significant effect on total ectomycorrhizal development or number of culls. Seedling density averaged 308/m<sup>2</sup> and was not affected by treatment. One Pt fruit body (5 cm in diameter and sessile) was detected at the end of the study in a plot of IMRD inoculum. Fruit bodies of Tt occurred on 3 percent of sampled seedlings; all naturally occurring ectomycorrhizae resembled those formed by this fungus.

*Hiwassee Land Company Nursery, Chatsworth, Georgia.*—During fumigation in this forest industry nursery, day air temperature (sunny) was 20°C and soil moisture was approximately 50 percent of field capacity. Plastic covering was removed after 5 days. Batch 3 inocula of IMRD and Abbott were stored for 7 and 12 days, respectively. Inoculum was applied and nontreated seeds of loblolly pine were planted on April 30, 1979. Seeds were covered with 1-cm layer of nonfumigated pine bark as a mulch. Midstudy root assessments were made in early August, and the study was terminated in January 1980. Seedlings were considered culls if shorter than 12 cm and with root-collar diameters less than 3 mm.

*Results:* Soil fumigation eliminated all organisms assayed (Table 9). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 68, 59, 16, and 16 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on 20 to 44 percent of short roots on control seedlings; no fruit bodies were observed at that time.

At termination of the study, seedlings that received IMRD and the highest rate of Abbott inocula had Pt indices of 57 and 59, respectively (Table 11). However, only Pt ectomycorrhizae from IMRD inoculum significantly increased seedling growth in comparison to control seedlings. None of the inoculum treatments had significant effects on total ectomycorrhizal development or number of culls. Seedling density averaged 487/m<sup>2</sup> and was not affected by treatment. Totals of 17, 18, 5, and 13 Pt fruit bodies were produced in plots of IMRD and the three decreasing Abbott inocula treatments, respectively. About half of these were stalked; diameters ranged from 1 to 6 cm. Most naturally occurring ectomycorrhizae resembled those formed by Tt. This fungus produced fruit bodies on 3 percent of the sampled seedlings.

*Vallonia State Nursery, Indiana.*—During soil fumigation, day air temperature (sunny) reached 20°C and soil moisture was moderate. Plastic covering was removed by strong winds after only 24 hours of fumigation. Batch 4 inocula of IMRD and Abbott were stored for 11 and 16 days, respectively, before use. Inoculum was applied and thiram-treated seeds of Virginia pine were planted on May 11, 1979. Seeds were covered with 2,240 kg/ha of hydromulch. Midstudy root assessments were made in late August, and the study was terminated in March 1980. Characteristics of cull seedlings were the same as in 1978.

*Results:* Even though the soil was covered with plastic for only 24 hours, soil fumigation eliminated all organisms assayed (Table 9). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices of 77, 77, 72, and 15 for IMRD and the three decreasing rates of Abbott inocula, respectively. Naturally

occurring ectomycorrhizae, resembling those formed by Tt, were observed on 16 to 36 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, all Pt inoculum treatments except the lowest rate of Abbott inoculum produced Pt indices between 51 and 66 on seedlings (Table 11). Pt ectomycorrhizae from the two highest rates of Abbott inoculum significantly increased seedling growth, and the IMRD and the highest rate of Abbott inocula significantly decreased seedling culls in comparison to control seedlings. All inoculum treatments significantly increased total ectomycorrhizal development. Seedling density averaged 317/m<sup>2</sup> and was not affected by treatment. Occurrence of Pt fruit bodies was not recorded. Tt fruit bodies occurred on more than 25 percent of sampled seedlings, and the naturally occurring ectomycorrhizae resembled those formed by this fungus.

## DISCUSSION

The four batches of IMRD inoculum produced high Pt indices in the IMRD Microplot Nursery and four of the five conventional nursery tests. The exception was the slash pine test in the Buckeye Cellulose Corporation Nursery. In three of the five nurseries, Pt ectomycorrhizae formed by this inoculum significantly increased seedling growth (total fresh weight) and, in one nursery, it significantly decreased the number of seedling culls. Thus, vegetative inoculum of Pt produced in 1979 at the IMRD was consistently effective. However, results of the inoculum characterization tests, determined before inoculum testing in the soil (Table 8), were not correlated with subsequent inoculum effectiveness. Batches of IMRD inoculum varied in moisture content from 6 to 53 percent and residual glucose levels from 4.6 to 6.7 mg/g. Also, only a few propagules of Pt were reisolated from batches of this highly effective inoculum whereas isolation revealed high levels of microbial contamination. Inoculum pH was the only consistent characteristic among batches of IMRD inoculum. Apparently, the peat moss component of the IMRD inoculum substrate effectively stabilized acidity between pH 4.6 and 5.0. The original objective of mixing different amounts of peat moss with vermiculite was to establish stable acid reactions to vermiculite substrates (Marx and Zak 1965). However, the ability of peat moss to lower and stabilize pH may not be the only benefit derived from using this component in production of viable Pt vegetative inoculum. Pt produces fulvic and humic acids in pure cultures (Tan and others 1978) and its vegetative growth in pure culture is stimulated by fulvic acid from the soil (Tan and Nopamornbodi 1979). Peat moss may be furnishing essential humic acids or their precursors to Pt much as organic matter does in forest soils. These humic acids may play an important role not only in the production of effective Pt inoculum but also in ectomycorrhizal development. Recently, a hot-water extract of peat moss was found to stimulate vegetative growth of Pt on agar medium (Marx, unpublished data).

Storage of IMRD inoculum for 4 weeks at 5°C significantly decreased the effectiveness of one of four inoculum batches (Table 10). Midstudy Pt indices were correlated with final indices, and generally Pt indices improved with time. The longest storage of IMRD inoculum in conventional nursery tests was 21 days for batch 1, tested on loblolly pine at the International Paper Company Nursery, it produced the highest Pt index (74) of all IMRD inocula tested in 1979.

The results obtained with different batches of Abbott inoculum in the IMRD Microplot Nursery test did not agree with the results obtained with these inocula in conventional nursery tests. Abbott inoculum batches 2, 3, and 4 were not effective in the microplot tests, regardless of rate or length of storage. However, they were highly effective when used at the higher rates in each conventional

nursery test. It is unfortunate that only a single test of each batch of Abbott inoculum was carried to completion. Results from additional nurseries would have furnished needed information to help explain this discrepancy. In the microplot tests, only Abbott batch 1 was effective for all storage periods with Pt indices as high as the IMRD inoculum. The high rate ( $2.16 \text{ l/m}^2$ ) of this inoculum was as effective in forming Pt ectomycorrhizae and fruit bodies as the IMRD inoculum ( $1.08 \text{ l/m}^2$ ) in both conventional nursery tests in which it was tested. In the International Paper Company Nursery, Pt ectomycorrhizae formed by Abbott batch 1 significantly increased seedling growth regardless of inoculum rate.

Pre-nursery test characterizations of Abbott and IMRD inocula did not furnish adequate information for predicting inoculum effectiveness. Abbott inoculum had more consistent moisture content, less microbial contamination, less residual glucose, and more Pt propagules, but a much higher pH than did the IMRD inocula (Table 8). Differences in characteristics between IMRD and Abbott inocula may be explained by handling techniques. After leaching, IMRD inoculum was dried in a low-humidity,  $20^\circ$  to  $26^\circ\text{C}$  drying room. The inoculum was placed 5 to 6 cm deep in 10-cm deep trays with screened bottoms and mixed manually every 2 to 4 hours for 90 to 100 continuous hours. It was consequently very difficult to accurately monitor moisture content of inoculum under these conditions. During the long drying period, the IMRD inoculum was exposed to a variety of airborne microorganisms which could explain the high levels of microbial contamination. This surface contamination which occurred long after Pt had permeated the vermiculite particles was apparently of no significant consequence to inoculum effectiveness. In comparison, the Abbott inoculum was dried in a forced dry-air apparatus, and drying required only a few hours. Leaching procedures appeared to have comparable results, since maximum glucose levels for IMRD and Abbott inocula were 6.7 and 6.5 mg/g, respectively.

Abbott Laboratories did not add peat moss to the 1979 inoculum (due to sterilization problems) which resulted in pH levels less acidic than the IMRD inoculum. The potential activity of the mycelium once added to nursery soil may have been significantly affected by inoculum pH. Abbott batch 1 was the most acidic and the most effective of all Abbott batches in both the IMRD and the two conventional nursery tests. Its pH (6.0) was similar to that of the soil in these three tests (differences of only 0.3 to 0.8 pH unit) and, therefore, the mycelium in this inoculum did not require physiological adjustment to broad pH ranges. Conversely, the other three batches of Abbott inoculum had considerably more alkaline reactions (average pH 7.2) and in the various tests were exposed to more extremes in soil pH (differences of 0.8 to 2.1 pH units). Although IMRD inoculum batch 1 (pH 5.0) used at the International Paper Company Nursery was placed in soil with inoculum:soil pH differences of 1.3 pH units, the fact that the inoculum was more acidic than the soil may have been a controlling factor in this physiological adjustment of Pt mycelium.

The 1979 formulation of Abbott inoculum was improved over that produced in 1977 and 1978. Only at the IMRD Microplot Nursery, did any Abbott batch equal the effectiveness of IMRD inoculum. Apparent discrepancies in quality control, production procedures, and inconsistent results between nurseries suggested that still more improvements in the fermentation procedures were needed at Abbott Laboratories.

#### 1980 TESTS

Apart from pH, the 1979 characterizations of inoculum assayed were of little value in predicting eventual effectiveness of inoculum in nurseries. A reliable method was needed whereby ectomycorrhizae would form on seedlings faster than

in the 6- to 8-month conventional nursery test or the 3- to 4-month, container-grown seedling test (Marx and others 1982).

#### FAST ASSAY AND INOCULUM CHARACTERIZATION

During the fall and winter of 1979–80, 12 different formulations of inoculum were produced by Abbott Laboratories and tested at the IMRD by various fast assay techniques for ectomycorrhizal synthesis. Nonmycorrhizal roots of loblolly pine seedlings grown in plastic pouches were inoculated according to the technique of Fortin and others (1980). An inoculum slurry-root dip technique described by Marx and others (1977b) was also tested. Both techniques utilize pine seedlings with abundant nonmycorrhizal roots developed for several weeks before inoculation. Both techniques produced abundant *Pt* ectomycorrhizae in 3 to 4 weeks. However, the inoculum slurry-root dip technique proved to be simpler and furnished more consistent results. The latter technique, used to assay subsequent inoculum batches, was as follows:

Loblolly pine seeds were sown in flats containing autoclaved vermiculite and grown in the mycorrhizal fungus-free growth room at the IMRD (Marx 1973) which received 75 percent of full sunlight for 10 to 11 hours daily. Seedlings were fertilized once after 5 weeks' growth with the equivalent of 5 ml/seedling of a 2 g/l solution of water-soluble NPK fertilizer (20-19-18). Ten weeks after germination, seedlings were carefully removed and their roots rinsed free of growing medium. Seedlings with at least three lateral roots 4 cm long, each supporting at least 10 short roots, were selected. Primary lateral roots and the taproot were trimmed to 8 cm. Roots of individual seedlings were immersed in a well-mixed, 1:1 (by volume) slurry of inoculum and water for 5 seconds. After draining for a few seconds, seedlings were transplanted into cavities of Hillson Rootainers® containing a 1:1 vermiculite:peat moss rooting medium. The containers were placed on a greenhouse bench and received 12 hours of full sunlight during each 24-hour period, supplemented with 4 hours of approximately 740 lumens/m<sup>2</sup> of incandescent light. Seedlings were watered three times a week and were not fertilized. Each batch of inoculum and control inoculum (1:1 vermiculite:peat moss) was replicated five times with eight seedlings per replicate in a randomized block design. After 25 days of growth, seedlings were removed from the containers and their roots rinsed free of growing medium. Roots were visually assessed for ectomycorrhizal development.

In earlier tests, certain batches of Abbott inoculum had a wide range of sizes of vermiculite particles (0.5 to 7 mm). This variation was suspected of limiting survival of *Pt* mycelium, especially in the smaller particles. Subsequently, another inoculum characterization assay was developed to determine the amount of viable *Pt* propagules in small particles. This assay involved screening inoculum through a screen with openings of 3.35 mm (No. 6) and placing 30 to 40 of the particles that collected on a screen with 2.36 mm openings (No. 8) on the surface of fortified MMN agar medium. These plates were incubated as previously described, and the percentage of particles yielding mycelial growth of *Pt* was recorded.

The results of the fast assay, and the other characterizations from the 12 formulations of Abbott inoculum, showed that peat moss was an important component of the initial inoculum substrate. If peat moss or acid buffers were added to the substrate of pure vermiculite inoculum after fermentation and drying, the inoculum was not as effective as that initially produced with peat moss. Also, inoculum with peat moss, if not leached with water before drying, was less effective in the fast assay than if leached and then dried. Leaching was found to remove over 60 percent of the glucose from the inoculum.

TABLE 12. Characteristics of vegetative inocula of *Pisolithus tinctorius* (Pt) produced by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories in 1980.

Inoculum	Bulk density (g/l)	Moisture content (percent)	Propagules Pt (Pt/g)	Pt from small particles (percent)	Residual glucose (mg/g)	pH	Contaminants	
							Bacteria dilution	Fungi dilution
IMRD	350	38	2	0	5.7	4.6	10 <sup>5</sup>	10 <sup>5</sup>
Abbott								
Batch 1	300	27	40	92	15.8	5.4 <sup>1</sup>	10 <sup>5</sup>	0
Batch 2	247	28	41	53	7.2	5.6 <sup>1</sup>	10 <sup>5</sup>	0
Batch 3	268	26	4	70	13.9	5.1 <sup>2</sup>	10 <sup>6</sup>	3 × 10 <sup>5</sup>

<sup>1</sup> 5 percent peat moss by volume with vermiculite in fermentor.

<sup>2</sup> 10 percent peat moss by volume with vermiculite in fermentor.

## MATERIALS AND METHODS

The most effective production procedures, as determined by the fast assay technique, were used to produce the 1980 Abbott inoculum. Three batches of Abbott inoculum were produced at weekly intervals by solid-substrate fermentation with vermiculite containing 5 to 10 percent peat moss by volume and routine nutrients. After steaming, the substrate was inoculated with twice as much starter mycelium of Pt (produced as in 1977 and 1978) as was used in the earlier tests and incubated. The inoculum was leached with water and then dried. IMRD inoculum was produced as in previous years. Characteristics of the 1980 inocula are presented in Table 12. IMRD inoculum and Abbott inocula batches 1, 2, and 3 were tested using the fast assay technique after 17, 16, 9, and 2 days of storage at 5°C, respectively.

In the initial characterization of IMRD inoculum (Table 12) very few propagules of Pt were recovered from inoculum particles in the two assays on MMN agar medium. This inoculum also had moderate levels of microbial contamination and residual glucose. The pH was 4.6, which was comparable to that of highly effective batches of IMRD inoculum reported earlier. In the characterization of Abbott inocula, batches 1 and 2 yielded high propagule counts of Pt from the agar medium assays and contained only moderate levels of bacterial contaminants and no fungal contaminants. Batch 1 had high levels of residual glucose, twice that of batch 2. Because of the addition of peat moss, the pH of these batches was more acidic than the previous Abbott inocula. Abbott batch 3 had low amounts of recoverable Pt propagules and more bacterial and fungus contaminants than batches 1 and 2. This batch also had more residual glucose than either Abbott batch 2 or IMRD inoculum, but it was more acidic than other Abbott batches, apparently due to the higher peat moss content.

The fast assay technique showed that all inocula were highly effective in forming Pt ectomycorrhizae on loblolly pine seedlings (Table 13). Nearly all ectomycorrhizae formed during the 25-day incubation period were those of Pt regardless of inoculum source or batch. This indicated that after 17 days of storage, all inocula contained viable mycelium of Pt capable of infecting roots and rapidly forming ectomycorrhizae under these conditions.

Since variation between batches of Abbott inoculum and variation in Pt ectomycorrhizae formed by the same batch in different nurseries were problems in

TABLE 13. *Ectomycorrhizal development on loblolly pine seedlings formed by various batches of Pisolithus tinctorius (Pt) inoculum produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories using the fast assay technique.*<sup>1</sup>

Inoculum source	Percent of seedlings with Pt ectomycorrhizae	Percent short roots ectomycorrhizal with—		Pt index <sup>2</sup>
		Pt	All fungi	
IMRD	100	68	70	97
Abbott				
Batch 1	100	74	75	99
Batch 2	100	63	65	97
Batch 3	100	53	55	95
Control	0	0	3	0

<sup>1</sup> Within columns there were no significant differences between means for IMRD and Abbott inocula.

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

previous tests, all batches of inoculum were examined in each nursery in 1980. Since routine commercial inoculum produced in the future will probably be a mixture of several batches, an even mixture of the three 1980 Abbott batches was also included in these tests. Inoculum rates of 1.08 and 0.27 l/m<sup>2</sup> were tested for each batch to ascertain the least amount of inoculum needed to obtain acceptable Pt indices.

Various fungicides stimulate Pt ectomycorrhizal development on container-grown pine seedlings (Pawuk and others 1980) and on pine seedlings grown in fumigated nursery soil (Marx and Rowan 1981). Certain fungicides, such as captan and benomyl, apparently enhance the efficacy of the Pt vegetative inoculum by depressing populations of competing and antagonistic microorganisms. A single application of captan (a soil fungicide used commonly in tree nurseries) was, therefore, an additional treatment variable in the 1980 nursery tests. The experimental design in each nursery test was six inoculum treatments (three batches Abbott inoculum singly and in an even mixture, one batch IMRD inoculum, and a pure vermiculite control)  $\times$  two inoculum (or vermiculite control) rates at 0.27 and 1.08 l/m<sup>2</sup> (plus a control without inoculum or vermiculite)  $\times$  two fungicide treatments (0 and 5.6 a.i. kg/ha of captan)  $\times$  five replicate blocks in a randomized block design. A total of 130 plots were involved in each nursery test. Twenty-six plots were spaced in each nursery bed (block) at the conventional nurseries and in microplots at the IMRD as was done in earlier tests. Experiments were installed as in earlier tests except soil in all plots not receiving inoculum (controls) was chopped to standardize soil manipulation and potential washing of seed by rain. This was done to minimize variation in seedling density between inoculated and noninoculated plots as experienced in earlier tests. After sowing of seeds, 1.4 g captan (50 percent WP) was applied by hand to designated plots as a drench in 3.2 l water/plot in the conventional nurseries and 0.35 g captan in 0.8 l water/plot at the IMRD.

In the IMRD Microplot Nursery and the conventional nurseries, the addition of vermiculite with and without captan did not significantly affect ectomycorrhizal development, seedling growth, or cull rate. Data from these vermiculite treatments are not presented.

TABLE 14. Number of Pythiaceae fungal propagules (pg) and plant parasitic nematodes isolated from pre- and post-fumigated soil samples in nursery tests in 1980.

Nursery	Pythiaceae fungi <sup>1</sup> pg/g		Nematodes/475 cc soil	
	Pre-fum	Post-fum	Pre-fum	Post-fum
IMRD, GA	8	0	12 <sup>2,3</sup>	0
Buckeye, FL	13	1	0 <sup>2</sup>	0
Champion, SC	7	0	1 <sup>2,4</sup>	0
Westvaco, SC	5	2	2 <sup>2,4</sup>	0

<sup>1</sup> Mainly *Pythium irregulare*.

<sup>2</sup> Saprophytic nematodes.

<sup>3</sup> Ring nematode (*Criconeimoides*).

<sup>4</sup> Stunt nematode (*Tylenchorhynchus*).

#### NURSERY INFORMATION AND RESULTS

Cropping history and cultural practices used in the 1980 nursery tests prior to installation of the study are given in Appendix X, chemical and physical characteristics of soil at installation of the study are given in Appendix XI, and cultural practices involved after installation of the study are given in Appendix XII; other details are as follows:

*IMRD Microplot Nursery, Georgia.*—Soil mixture, soil fumigation, and fertilization, as well as seeding and maintenance of plots were done as in 1979. IMRD inoculum and Abbott inocula batches 1, 2, and 3 were stored for 24, 23, 16, and 9 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 8, 1980. Fumigated pine straw was placed 1 cm deep over seeds as a mulch. Midstudy root assessments of ectomycorrhizae were done in late August, and the study was terminated in March 1981. Characteristics of seedling culls were the same as in 1977.

*Results:* Soil fumigation eliminated all organisms assayed (Table 14). Midstudy assessments of ectomycorrhizae on the seedlings revealed that both rates of IMRD inoculum and the highest rate of batches 1 and 2 of Abbott inocula produced Pt indices >50; there was no discernible effect of captan (Table 15). Naturally occurring ectomycorrhizae, resembling those formed by Tt, were found on more than 20 percent of the short roots on control seedlings at that time.

At termination of the study, IMRD and Abbott batches 1 and 2 at both rates and the mixture of Abbott inocula at the highest rate, with and without captan, produced Pt indices >50 on the loblolly pine seedlings (Table 15). The Abbott mixture at the lowest rate with captan produced a Pt index of 63. Abbott batch 3 was ineffective at any rate, with or without captan. In both fungicide treatments, IMRD inoculum at the lowest rate was more effective in forming Pt ectomycorrhizae and fruit bodies than the lowest rate of Abbott inocula, with or without captan. Rate of IMRD inoculum did not affect its ability to form Pt ectomycorrhizae, but more fruit bodies were produced at the highest rate. The highest rate of Abbott batches 1 and 2 and the mixture without captan were more effective than the lowest rate of the same inoculum batches without captan. However, the lowest rate of these inoculum batches with captan produced Pt indices similar to those produced by the highest rate of these inocula without captan, thus demonstrating the stimulating effects of captan.

Midstudy Pt indices from all treatments were generally lower than final indices, indicating that Pt spread to more roots during the last half of the growing season.

TABLE 15. *Ectomycorrhizal development of loblolly pine seedlings from the IMRD Microplot Nursery, Georgia, at midstudy and final assessment after treatment with vegetative inocula of Pisolithus tinctorius (Pt) produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and with a captan treatment.*<sup>1</sup>

Inoculum rate and captan treatment	Midstudy assessment				Final assessment				Total number Pt fruiting bodies
	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	
	Pt	All fungi			Pt	All fungi			
IMRD									
1.08 l/m <sup>2</sup>									
None	54a	59a	100a	92a	63a	69a	100a	91a	21a
Captan	58a	63a	100a	93a	63a	70a	100a	89a	29a
IMRD									
0.27 l/m <sup>2</sup>									
None	48a	53a	96a	86ab	64a	71a	100a	89a	15b
Captan	33ab	41ab	88ab	72b	65a	71a	100a	91a	13b
Abbott batch 1									
1.08 l/m <sup>2</sup>									
None	30ab	40ab	68bc	50c	47b	57ab	100a	78ab	5c
Captan	26b	35b	80ab	59c	65a	71c	100a	92a	11b
Abbott batch 1									
0.27 l/m <sup>2</sup>									
None	14c	29d	60c	28d	30bc	48b	80b	52c	2c
Captan	17bc	30bc	40d	26d	37bc	53ab	98a	69bc	4c
Abbott batch 2									
1.08 l/m <sup>2</sup>									
None	27b	38b	68bc	48c	47b	60a	92ab	72ab	10b
Captan	40a	51a	76b	62bc	65a	72a	100a	90a	5c
Abbott batch 2									
0.27 l/m <sup>2</sup>									
None	13c	31bc	40d	25d	29c	47b	80b	52c	1d
Captan	8c	27d	28e	9e	38bc	54ab	80b	61bc	7c
Abbott batch 3									
1.08 l/m <sup>2</sup>									
None	0	27d	0	0	1d	38c	14d	2e	0
Captan	1d	23d	4f	<1f	0	38c	0	0	0
Abbott batch 3									
0.27 l/m <sup>2</sup>									
None	1d	22d	4f	<1f	1d	32c	8d	1e	0
Captan	1d	26d	6f	<1f	1d	39c	6d	1e	0
Abbott mixture									
1.08 l/m <sup>2</sup>									
None	18bc	30bc	56c	34d	36bd	52ab	92ab	63bc	5c
Captan	10c	22d	68bc	32d	46b	58ab	100a	77ab	3c
Abbott mixture									
0.27 l/m <sup>2</sup>									
None	5d	24d	26e	5e	23d	38c	70c	42d	3c
Captan	5d	27a	24c	4e	36bc	51ab	92ab	63bc	6c

TABLE 15. Continued.

Inoculum rate and captan treatment	Midstudy assessment				Final assessment				Total number Pt fruiting bodies
	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	
	Pt	All fungi			Pt	All fungi			
Control									
None	0	23d	0	0	0	31	0	0	0
Captan	0	19d	0	0	0	30	0	0	0
Overall effect <sup>3</sup>									
None	22	34	52	37	34	51	74	54	62
Captan	20	32	51	36	42*	55	78	63*	78*

<sup>1</sup> Means sharing a common letter in the same column are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> \* Denotes significantly different overall mean effect of captan treatment.

Captan improved inoculum effectiveness regardless of source or batch. In all plots drenched with captan, seedlings had greater ectomycorrhizal development, the Pt indices were higher, and more Pt fruit bodies were produced. Regardless of treatment, most fruit bodies were sessile and averaged 3.1 cm in diameter. Fruit bodies of Tt, the primary naturally occurring ectomycorrhizal fungus in this test, were present on nearly 12 percent of sampled seedlings; most occurred on seedlings in captan-treated, Abbott batch 3 and control plots.

Seedling height, root-collar diameter, and top and root fresh weights averaged 22.1 cm, 5.0 mm, and 7.4 and 3.4 g, respectively, and were not significantly affected by treatment in comparison to control seedlings. Seedling culls averaged 24 percent, and seedling density averaged 285/m<sup>2</sup>; neither parameter was affected by treatment. These data, therefore, are not presented.

*Buckeye Cellulose Corporation Nursery, Florida.*—During fumigation, soil conditions were the same as in 1977. IMRD inoculum and Abbott inocula batches 1, 2, and 3 were stored for 31, 30, 23, and 16 days, respectively, before use. Inoculum was applied and nontreated seeds of slash pine were planted on April 15, 1980. Seeds were covered with 1,120 kg/ha of hydromulch. Midstudy root assessments were made in early September, and the study was terminated in January 1981. Characteristics of seedling culls were the same as 1979 except seedlings with pitch canker were culled also. Incidence of pitch canker (Blakeslee and others 1980) caused by *Fusarium moniliforme* var. *subglutinans*, was determined in November 1980<sup>3</sup> in each plot to ascertain the effect of Pt inoculation and captan on disease incidence.

*Results:* Soil fumigation eliminated all but a few Pythiaceae fungi (Table 14). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices

<sup>3</sup> Disease assessments made by G. M. Blakeslee, School of Forest Resources, University of Florida, Gainesville 32611, and T. H. Miller, USDA Forest Service, Southeastern Forest Experiment Station, School of Forest Resources, University of Florida, Gainesville, 32611.

TABLE 16. *Ectomycorrhizal development of slash pine seedlings from the Buckeye Cellulose Corporation Nursery, Florida, at midstudy and final assessment after treatment with vegetative inocula of Pisolithus tinctorius (Pt) produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and with a captan treatment.*<sup>1</sup>

Inoculum rate and captan treatment	Midstudy assessment				Final assessment				Total number Pt fruiting bodies
	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	
	Pt	All fungi			Pt	All fungi			
IMRD									
1.08 l/m <sup>2</sup>									
None	58a	69a	100a	84a	22b	30ab	98ab	68ab	17a
Captan	48ab	62a	100a	77ab	22b	30ab	100a	73ab	25a
IMRD									
0.27 l/m <sup>2</sup>									
None	38b	60a	96ab	61bc	18bc	29bc	88ab	56b	11ab
Captan	40ab	58a	96ab	66b	17bc	27bc	98ab	60ab	19a
Abbott batch 1									
1.08 l/m <sup>2</sup>									
None	52a	64a	100a	81a	28a	36ab	98ab	74ab	9ab
Captan	51a	63a	100a	82a	32a	41a	98ab	73ab	6b
Abbott batch 1									
0.27 l/m <sup>2</sup>									
None	38b	58a	96ab	63b	16bc	27bc	94ab	53b	8ab
Captan	54a	68a	96ab	76ab	23b	33ab	98ab	68ab	7ab
Abbott batch 2									
1.08 l/m <sup>2</sup>									
None	50a	62a	100a	81a	26ab	44a	98ab	71ab	14ab
Captan	54a	62a	100a	82a	26ab	36ab	100a	69ab	6b
Abbott batch 2									
0.27 l/m <sup>2</sup>									
None	21c	57a	72c	27d	15bc	26bc	88ab	51b	1b
Captan	54a	68a	96ab	76ab	18bc	33ab	94ab	56b	4b
Abbott batch 3									
1.08 l/m <sup>2</sup>									
None	28c	56a	80bc	40cd	10c	25bc	86ab	32c	9ab
Captan	35b	58a	88b	53c	17bc	27bc	94ab	59ab	9ab
Abbott batch 3									
0.27 l/m <sup>2</sup>									
None	11c	54ab	52d	11d	6c	22c	52c	17c	3b
Captan	16c	54ab	48d	14d	4c	25bc	54c	12c	1b
Abbott mixture									
1.08 l/m <sup>2</sup>									
None	52a	64a	92ab	75ab	30a	38ab	100a	78a	6b
Captan	56a	68a	100a	82a	26ab	34ab	100a	74ab	5b
Abbott mixture									
0.27 l/m <sup>2</sup>									
None	30bc	55ab	76c	41cd	14c	25bc	92ab	51b	5b
Captan	40ab	60a	96ab	64b	17bc	27bc	84b	52b	8ab

TABLE 16. Continued.

Inoculum rate and captan treatment	Midstudy assessment				Final assessment				Total number Pt fruiting bodies
	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	
	Pt	All fungi			Pt	All fungi			
Control									
None	0	46b	0	0	0	23c	0	0	0
Captan	0	49b	0	0	0	21c	0	0	0
Overall effect <sup>3</sup>									
None	30	60	86	56	19	28	89	55	83
Captan	45*	62	92	67*	20	29	92	60*	90

<sup>1</sup> Means sharing a common letter in the same column are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> \* Denotes significantly different overall mean effect of captan treatment.

> 50 for all IMRD inoculum treatments, captan-treated Abbott batch 2 and mixture treatments at the lowest rate, and Abbott batch 3 at the highest rate (Table 16). Stimulation of Pt ectomycorrhizal development by captan was evident in these latter three treatments. Naturally occurring ectomycorrhizae, resembling those formed by Tt and *R. nigrescens*, were abundant on control seedlings; fruit bodies of both fungi were observed in a few plots at that time.

Incidence of pitch canker on seedlings was not affected by inoculum treatment or the interactions of inoculum treatments with captan. Overall, however, captan significantly reduced the incidence of pitch canker from an average of 0.43 percent for treatments without captan to an average of 0.26 percent for treatments with captan.

At termination of the study, seedlings that received IMRD and Abbott batches 1 and 2 and the mixture of Abbott inoculum at both rates with and without captan and batch 3 at the highest rate with captan had Pt indices > 50 on seedlings (Table 16). There were no significant differences between final Pt indices of these Abbott inoculum treatments and those of IMRD inoculum treatments. Numerically, the highest Pt index was obtained with the mixture treatment of Abbott inoculum at the highest rate without captan. It was higher than that of IMRD, Abbott batches 1 and 2 at the lowest rates without captan, Abbott batch 3 at the highest rate without captan and the lowest rate with both captan treatments. A Pt index > 50 was not obtained with batch 3 of Abbott inoculum, except at the highest rate with captan. Although not always significant, there were generally more Pt ectomycorrhizae, higher Pt indices, and more Pt fruit bodies produced at the highest rate of inoculum regardless of source. With the exception of the highest rate of batch 3 of Abbott inoculum, captan did not increase Pt ectomycorrhizal development in a given inoculum treatment. But when it was averaged over all the inocula, it significantly increased the percentage of short roots with Pt ectomycorrhizae as well as Pt indices. Fruit bodies of Pt were produced in all inoculum and captan treatments; most were produced by IMRD inoculum. More than 90 percent of the 173 fruit bodies were sessile; the average diameter was 4.2 cm.

Midstudy assessments of ectomycorrhizae revealed nearly twice the number

TABLE 17. *Ectomycorrhizal development of loblolly pine seedlings from Champion-International Corporation Nursery, South Carolina, at midstudy and final assessment after treatment with vegetative inocula of Pisolithus tinctorius (Pt) produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and with a captan treatment.*<sup>1</sup>

Inoculum rate and captan treatment	Midstudy assessment				Final assessment			
	Percent short roots ectomy-corrhizal with—		Percent seed-lings with Pt	Pt index <sup>2</sup>	Percent short roots ectomy-corrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>
	Pt	All fungi			Pt	All fungi		
IMRD								
1.08 l/m <sup>2</sup>								
None	24a	31a	60a	44b	32ab	47ab	100a	66ab
Captan	30a	39a	80a	62a	40a	55a	100a	71a
IMRD								
0.27 l/m <sup>2</sup>								
None	11b	34a	60a	19c	20ab	40bc	85ab	44bc
Captan	18a	32a	50a	28c	18bc	38bc	83ab	38c
Abbott batch 1								
1.08 l/m <sup>2</sup>								
None	16a	36a	45ab	20c	24ab	43bc	98ab	53bc
Captan	10b	28a	30b	11c	16bc	34bc	93ab	42bc
Abbott batch 1								
0.27 l/m <sup>2</sup>								
None	9b	30a	15c	5d	14bc	32c	85ab	39c
Captan	0	18b	0	0	19bc	30c	90ab	55ab
Abbott batch 2								
1.08 l/m <sup>2</sup>								
None	18a	36a	35b	18c	23ab	41bc	90ab	52bc
Captan	11b	27a	25bc	10c	21ab	38bc	100a	55ab
Abbott batch 2								
0.27 l/m <sup>2</sup>								
None	5c	21b	20c	5d	16bc	36bc	83ab	38c
Captan	4c	19b	15c	3d	17bc	32c	83ab	44bc
Abbott batch 3								
1.08 l/m <sup>2</sup>								
None	0	20b	0	0	0	38bc	0	0
Captan	0	24b	0	0	9c	40bc	33d	12d
Abbott batch 3								
0.27 l/m <sup>2</sup>								
None	0	23b	0	0	0	35bc	0	0
Captan	1c	25b	5c	<1d	1d	43bc	8d	1e
Abbott mixture								
1.08 l/m <sup>2</sup>								
None	13ab	32a	25bc	10c	22ab	38bc	95ab	53bc
Captan	12b	30a	30b	12c	22ab	34bc	100a	61a
Abbott mixture								
0.27 l/m <sup>2</sup>								
None	3c	19b	15c	2d	11c	34bc	73bc	24cd
Captan	3c	31a	10c	1d	17bc	37bc	68c	30c

TABLE 17. Continued.

Inoculum rate and captan treatment	Midstudy assessment				Final assessment			
	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	Percent short roots ectomy- corrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>
	Pt	All fungi			Pt	All fungi		
Control								
None	0	16b	0	0	0	36bc	0	0
Captan	0	21b	0	0	0	34bc	0	0
Overall effect <sup>3</sup>								
None	10	27	28	12	16	38	71	37
Captan	9	27	25	13	18	38	76	41*

<sup>1</sup> Means sharing a common letter in the same column are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> \* Denotes significantly different overall mean effect of captan treatment.

observed at final assessment. During midstudy assessment the small seedlings were easily removed from the soil, resulting in minimal root damage. During the final assessment, larger seedling size increased the difficulty of removing intact root systems, consequently many ectomycorrhizae were stripped from the roots during final lifting. The primary naturally occurring ectomycorrhizae resembled those formed by Tt and *R. nigrescens*. Fruit bodies of both fungi were abundant in all test plots.

Seedling height, root-collar diameter, top and root fresh weights averaged 22.6 cm, 4.8 mm, 10.5 and 3.0 g, respectively, and were not significantly affected by treatment. Seedling culls averaged 14 percent, and seedling density averaged 327/m<sup>2</sup>; neither parameter was affected by treatments. These nonsignificant data are not presented.

*Champion-International Corporation Nursery, South Carolina.*—During fumigation, soil and temperature conditions were similar to those in 1979. IMRD inoculum and Abbott inocula batches 1, 2, and 3 were stored for 34, 33, 26, and 19 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 18, 1980. Seeds were covered with 1,792 kg/ha of hydromulch. Midstudy root assessments were made in early September, and the study was terminated in February 1981. Characteristics of seedling culls were the same as in 1979.

*Results:* Soil fumigation eliminated all organisms assayed (Table 14). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices <50 in all treatments except those of the highest rate of IMRD inoculum. Captan had no detectable effect at this time except on the highest rate of IMRD (Table 17). Naturally occurring ectomycorrhizae, resembling those formed by Tt, were observed on 16 to 25 percent of short roots on control seedlings; no fruit bodies were detected at that time.

At termination of the study, seedlings that received the high rate of IMRD inoculum with and without captan had Pt indices >50; the low rate did not (Table 17). Abbott batches 1 and 2 and the mixture of Abbott inoculum at the highest rate produced Pt indices >50 and, with the exception of batch 1, did so with and

TABLE 18. Growth of loblolly pine seedlings from the Champion-International Paper Company Nursery, South Carolina, with vegetative inocula of *Pisolithus tinctorius* (Pt) produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and with a captan treatment.<sup>1</sup>

Inoculum rate and captan treatment	Height (cm)	Root collar diameter (mm)	Fresh weight (g)		
			Top	Root	Total
IMRD					
1.08 l/m <sup>2</sup>					
None	23.5a <sup>2</sup>	5.3a	11.7a	4.5a	16.2a
Captan	21.5abc	4.9bc	9.3bc	4.1ab	13.5ab
IMRD					
0.27 l/m <sup>2</sup>					
None	21.4abc	5.0ab	9.5bc	3.7ab	13.2bc
Captan	23.0ab	5.0ab	9.8ab	3.6ab	13.4ab
Abbott batch 1					
1.08 l/m <sup>2</sup>					
None	20.3c	4.5c	8.1cd	3.5bc	11.6bc
Captan	22.0ab	4.9bc	9.9ab	3.4bc	13.3bc
Abbott batch 1					
0.27 l/m <sup>2</sup>					
None	20.7c	4.8bc	9.1bc	3.7ab	12.8bc
Captan	20.3c	4.5c	7.7d	3.0c	10.7c
Abbott batch 2					
1.08 l/m <sup>2</sup>					
None	20.4c	4.8bc	9.2bc	4.0ab	13.2bc
Captan	23.1ab	4.6bc	8.3cd	3.1bc	11.4bc
Abbott batch 2					
0.27 l/m <sup>2</sup>					
None	21.2abc	4.8bc	9.2bc	3.5bc	12.7bc
Captan	21.7abc	4.7bc	9.2bc	3.5bc	12.7bc
Abbott batch 3					
1.08 l/m <sup>2</sup>					
None	22.6ab	5.0ab	10.0ab	3.4bc	13.4ab
Captan	22.5ab	4.5bc	8.4cd	3.0bc	11.4bc
Abbott batch 3					
0.27 l/m <sup>2</sup>					
None	21.8abc	5.0ab	9.0bc	3.5bc	12.5bc
Captan	20.6c	4.8bc	9.4bc	3.6ab	13.0bc
Abbott mixture					
1.08 l/m <sup>2</sup>					
None	20.5c	4.8bc	8.4cd	3.6ab	12.0bc
Captan	23.4ab	5.2ab	10.7ab	3.9ab	14.6ab
Abbott mixture					
0.27 l/m <sup>2</sup>					
None	22.1ab	4.6bc	8.5cd	3.3bc	11.8bc
Captan	22.9ab	5.2ab	10.1ab	4.0ab	14.1ab
Control					
None	21.1abc	4.9bc	9.0bc	3.5bc	12.5bc
Captan	22.3ab	4.8bc	9.3bc	3.3bc	12.6bc

TABLE 18. *Continued.*

Inoculum rate and captan treatment	Height (cm)	Root collar diameter (mm)	Fresh weight (g)		
			Top	Root	Total
Overall effect <sup>3</sup>					
None	21.4	4.9	9.2	3.7	12.9
Captan	22.1	4.8	9.3	3.5	12.8

<sup>1</sup> Means sharing a common letter in the same column are not significantly different at  $P = 0.05$ .

<sup>2</sup> Seedling tops were mowed to 20 cm height twice during growing season.

<sup>3</sup> There was no significant overall effect of captan on seedling growth.

without captan. At the lowest rate only Abbott batch 1 with captan produced a Pt index >50. Abbott batch 3 was ineffective in all treatments. Although many Pt fruit bodies were observed, their numbers were not recorded. Naturally occurring ectomycorrhizae resembled those formed by Tt, and its fruit bodies occurred on nearly 30 percent of all sampled seedlings. Captan did not consistently affect Pt ectomycorrhizal development in any inoculum treatment but, when averaged over all inocula, it significantly improved final Pt indices.

Midstudy Pt indices were lower than final Pt indices in all treatments. The significance of this to predictability of inoculum effectiveness has already been discussed.

Seedling growth was influenced by Pt inoculum and captan treatments but consistent patterns were not evident (Table 18). The largest seedlings (total fresh weight) were from the highest rate of IMRD inoculum which also produced the highest Pt index. However, the correlation between fresh weights and Pt indices of seedlings from other inoculum treatments were variable. The effect of captan was inconsistent. Seedling growth parameters were increased by captan in certain inoculum treatments but decreased in others. The effect of captan on seedling growth averaged over all inocula was not significant. Seedling density averaged 352/m<sup>2</sup>, and seedling culls averaged 15 percent; neither parameter was affected by treatment.

*Westvaco Corporation Nursery, South Carolina.*—Soil conditions during fumigation were not monitored. IMRD inoculum and Abbott inocula batches 1, 2, and 3, were stored for 41, 40, 33, and 26 days, respectively, before use. Inoculum was applied and thiram-treated seeds of loblolly pine were planted on April 25, 1980. Seeds were covered with 2,912 kg/ha of hydromulch. Midstudy root assessments were made in early September, and the study was terminated in January 1981. Characteristics of seedling culls were the same as in 1979.

*Results:* Soil fumigation eliminated all but a trace of Pythiaceae fungi (Table 14). Midstudy assessments of ectomycorrhizae on the seedlings revealed Pt indices between 65 and 90 for the IMRD inoculum treatments, but variable indices between <1 and 47 for Abbott inoculum treatments; Abbott batch 3 was ineffective (Table 19). The captan treatment increased the overall number of seedlings with Pt ectomycorrhizae and Pt indices for several Abbott inoculum treatments. About 70 percent of the naturally occurring ectomycorrhizae on all control seedlings resembled those formed by Tt; the remaining were the jet-black ectomycorrhizae formed by *C. geophilum*.

At termination of the study, seedlings that received both rates of IMRD inoculum, the high rate of Abbott batches 1 and 2, and the mixture treatments with and without captan produced Pt indices >50. The low rate of all Abbott inocula

TABLE 19. Ectomycorrhizal development of loblolly pine seedlings from the Westvaco Corporation Nursery, South Carolina, at midstudy and final assessment after treatment with vegetative inocula of *Pisolithus tinctorius* (Pt) produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and with a captan treatment.<sup>1</sup>

Inoculum rate and captan treatment	Midstudy assessment				Final assessment				Total number Pt fruiting bodies
	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	Percent short roots ectomycorrhizal with—		Percent seedlings with Pt	Pt index <sup>2</sup>	
	Pt	All fungi			Pt	All fungi			
IMRD									
1.08 l/m <sup>2</sup>									
None	45a	51a	96a	85a	45a	57a	100a	80a	34a
Captan	47a	52a	100a	90a	44a	54ab	100a	80a	21ab
IMRD									
0.27 l/m <sup>2</sup>									
None	23b	31bc	88ab	65b	35ab	50ab	96ab	66ab	14b
Captan	35ab	41b	80ab	68b	43a	57a	100a	75ab	24ab
Abbott batch 1									
1.08 l/m <sup>2</sup>									
None	16c	28bc	36d	21cd	30ab	49bc	84bc	53bc	21ab
Captan	24b	33bc	64c	47c	27bc	44bc	30de	54bc	9b
Abbott batch 1									
0.27 l/m <sup>2</sup>									
None	12c	18c	24d	16e	18c	48bc	44d	26cd	3c
Captan	5d	17c	16d	5e	9d	43bc	30de	14cd	2c
Abbott batch 2									
1.08 l/m <sup>2</sup>									
None	16c	25c	40c	26cd	23c	44bd	90ab	50bc	5c
Captan	26b	34bc	52c	40c	32ab	50ab	92ab	58ab	12b
Abbott batch 2									
0.27 l/m <sup>2</sup>									
None	7c	18c	24d	9e	10cd	40bc	46d	19cd	2c
Captan	7c	20c	25d	9e	6d	41bc	18e	7d	0
Abbott batch 3									
1.08 l/m <sup>2</sup>									
None	4d	17c	16d	4e	1d	36cd	2e	1d	0
Captan	8c	21c	20d	8e	5d	40bc	14e	7d	1c
Abbott batch 3									
0.27 l/m <sup>2</sup>									
None	1d	14c	4e	<1f	0	37cd	0	0	0
Captan	1d	15c	4e	<1f	2d	39cd	8e	2d	2c
Abbott mixture									
1.08 l/m <sup>2</sup>									
None	20bc	30bc	40c	27cd	28bc	44bc	79c	50bc	10b
Captan	16c	24c	52c	35cd	33ab	52ab	76c	52bc	10b
Abbott mixture									
0.27 l/m <sup>2</sup>									
None	5c	21c	20d	5e	7d	40bc	24e	10d	0
Captan	9c	21c	24d	10e	14d	43bc	44d	20cd	2c

TABLE 19. Continued.

Inoculum rate and captan treatment	Midstudy assessment				Final assessment				Total number Pt fruiting bodies
	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	Percent short roots ectomy- corrhizal with—		Percent seed- lings with Pt	Pt index <sup>2</sup>	
	Pt	All fungi			Pt	All fungi			
Control									
None	0	18c	0	0	0	38cd	0	0	0
Captan	0	13c	0	0	0	35d	0	0	0
Overall effect <sup>3</sup>									
None	15	25	37	26	20	44	57	31	89
Captan	18	26	44*	31*	22*	45	57	37*	83

<sup>1</sup> Means sharing a common letter in the same column are not significantly different at  $P = 0.05$ .

<sup>2</sup> Pt index =  $a \times (b/c)$  where  $a$  = percent of seedlings with Pt ectomycorrhizae,  $b$  = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and  $c$  = average percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

<sup>3</sup> \* Denotes significantly different overall mean effect of captan treatment.

treatments and neither rate of Abbott batch 3 produced Pt indices >50. Captan did not improve the effectiveness of these latter inoculum treatments. However, overall, captan improved the percentage of short roots ectomycorrhizal with Pt and improved Pt indices. All inoculum treatments produced Pt fruit bodies but most were produced by IMRD inoculum; captan did not influence the incidence of fruit bodies. Over 80 percent of the fruit bodies were sessile; average diameter of all fruit bodies was 3.9 cm. The highest rate of IMRD inoculum produced four to five Pt fruit bodies per m<sup>2</sup> of nursery soil during the growing season. Pt fruit bodies were present on 14 percent of the seedlings and most naturally occurring ectomycorrhizae resembled those formed by it. A low but consistent amount of *C. geophilum* ectomycorrhizae (2 percent) was also observed on seedlings regardless of treatment. In most instances, seedlings with low Pt indices at midstudy generally had higher indices at final assessment. Seedlings with high Pt indices at midstudy, however, had similar indices at final assessment.

Seedling growth was significantly affected by treatment (Table 20). In comparison to control seedlings significant growth increases and reduction of culls were associated with the higher Pt indices (Table 19). The four treatments of IMRD inoculum that produced the highest Pt indices (mean of 77) reduced seedling culls by more than three times in comparison to control seedlings. Captan did not affect growth of seedlings in many inoculum treatments but, overall, it did improve total fresh weight of seedlings, which also was associated with an overall higher Pt index. Seedling density was not affected by treatment but was very low (116/m<sup>2</sup>) because of poor seed germination.

## DISCUSSION

Vegetative inoculum of Pt produced in industrial fermentors in 1980 with new procedures (i.e., peat moss added to substrate and inoculum leached before drying) was capable of consistently forming abundant Pt ectomycorrhizae on pine seedlings in these nursery tests. However, initial characterizations of the Abbott inocula and results of the fast assay failed to correlate with the results obtained in the nurseries. IMRD inoculum produced very few Pt propagules on agar medium

TABLE 20. Growth of loblolly pine seedlings from the Westvaco Nursery, South Carolina, with vegetative inocula of *Pisolithus tinctorius* (Pt) produced in 1980 by the Institute for Mycorrhizal Research and Development (IMRD) and Abbott Laboratories, and with a captan treatment.<sup>1</sup>

Inoculum rate and captan treatment	Height (cm)	Root diameter (mm)	Fresh weight (g)			Percent culls
			Top	Root	Total	
IMRD						
1.08 l/m <sup>2</sup>						
None	27.3a <sup>2</sup>	7.3a	22.4a	12.5ab	34.9ab	12d
Captan	27.3a	7.8a	25.2a	14.2a	39.4a	9d
IMRD						
0.27 l/m <sup>2</sup>						
None	25.9ab	6.8ab	18.8b	10.9ab	29.7bc	12d
Captan	26.0ab	6.7ab	18.2b	9.9bc	28.1bc	14d
Abbott batch 1						
1.08 l/m <sup>2</sup>						
None	24.0abc	6.2ab	15.1cd	8.9cd	24.0cd	30b
Captan	22.8c	5.8bc	14.0d	9.2bc	23.2cd	38b
Abbott batch 1						
0.27 l/m <sup>2</sup>						
None	23.9bc	6.2ab	16.1bc	10.8ab	26.9bc	37ab
Captan	21.5cd	5.8bc	12.9e	8.5cd	21.4cd	42ab
Abbott batch 2						
1.08 l/m <sup>2</sup>						
None	21.5cd	5.8bc	12.8e	9.0bc	21.8cd	36ab
Captan	24.9ab	6.5ab	16.7bc	11.4ab	28.1bc	44ab
Abbott batch 2						
0.27 l/m <sup>2</sup>						
None	22.5c	5.9bc	12.7e	8.1cd	20.8cd	39ab
Captan	19.0d	5.8bc	13.4d	9.1bc	22.5cd	43ab
Abbott batch 3						
1.08 l/m <sup>2</sup>						
None	23.0bc	5.6bc	12.6e	8.0cd	20.6cd	39ab
Captan	22.0c	5.9bc	12.7e	9.1bc	21.8cd	46a
Abbott batch 3						
0.27 l/m <sup>2</sup>						
None	23.8bc	6.2ab	15.1cd	10.0ab	25.1cd	36ab
Captan	24.0bc	6.6ab	16.7bc	10.3ab	27.0bc	46a
Abbott mixture						
1.08 l/m <sup>2</sup>						
None	21.7cd	5.7bc	12.0e	8.0cd	20.0cd	49a
Captan	24.6ab	6.4ab	16.4bc	10.1ab	26.5bc	27c
Abbott mixture						
0.27 l/m <sup>2</sup>						
None	22.6c	5.8bc	13.5d	8.9cd	22.3cd	32ab
Captan	23.2bc	5.9bc	13.6d	8.3cd	21.9cd	40ab
Control						
None	21.8cd	5.3c	10.6e	6.4d	17.0d	53a
Captan	22.1c	5.8bc	13.2de	8.6cd	21.8cd	50a

TABLE 20. *Continued.*

Inoculum rate and captan treatment	Height (cm)	Root diameter (mm)	Fresh weight (g)			Percent culls
			Top	Root	Total	
Overall effect <sup>3</sup>						
None	23.5	6.1	14.7	9.2	23.9	34
Captan	23.4	6.3	15.7	9.9	25.6*	37

<sup>1</sup> Means sharing a common letter in the same column are not significantly different at  $P = 0.05$ .

<sup>2</sup> Seedling tops were mowed to 20 cm height twice during growing season.

<sup>3</sup> \* Denotes significantly different overall mean effect of captan treatment.

and had a high microbial contamination level. This inoculum, however, was highly effective in forming *Pt* ectomycorrhizae in all tests. Conversely, Abbott inoculum batches 1 and 2 had high *Pt* propagule counts, no fungal contamination, and were also highly effective in forming *Pt* ectomycorrhizae in most tests. Low effectiveness of Abbott batch 3 was more predictable based on certain of its initial characteristics. It produced few *Pt* propagules in one agar medium test, had the most microbial contamination of all inocula associated with a high residual glucose level, and was least effective in forming *Pt* ectomycorrhizae in all 1980 nursery tests. However, it was very effective in the fast assay. Obviously, viable hyphae of *Pt* were present in this inoculum that could be reisolated on agar medium and produce a positive result in the fast assay. Most of the hyphae, however, were external to the vermiculite particles as confirmed by microscopic examination. External hyphae may grow well on agar medium and also be capable of rapidly infecting preformed short roots in the fast assay. External hyphae, however, may fail to survive the rigors of exposure in soil where they must survive for at least 6 weeks after seeding before short-root development occurs on seedlings. Most external hyphae in the IMRD inoculum were destroyed during leaching, prolonged drying, and the several days of storage before testing at Abbott Laboratories, which accounts for its low *Pt* propagule counts. The positive results obtained with IMRD inoculum in the various *Pt* ectomycorrhizae synthesis tests suggest, therefore, that the most viable hyphae in inoculum which is effective in ectomycorrhizal development in nurseries is inside the vermiculite particles. All inocula which produced *Pt* indices >50 in the 1980 tests had abundant hyphae inside the vermiculite particles.

The acidity of the Abbott inoculum was improved by the addition of peat moss. The amount of residual glucose in inoculum did not appear to be strongly related to inoculum effectiveness. IMRD inoculum with the least amount of residual glucose had effectiveness comparable to Abbott batch 1 with nearly three times more glucose. These data, however, do not suggest that inoculum should not be leached. They simply indicate that, even after leaching, variable amounts of glucose and, undoubtedly, other nutrients still remain in the inoculum.

Captan failed to stimulate *Pt* ectomycorrhizal development in all inoculum treatments. However, when the captan effects were averaged for all inocula, it increased inoculum effectiveness. It had a greater effect on inocula applied at the low rates than at higher rates. This latter observation was most evident at the IMRD Microplot Nursery and the Buckeye Cellulose Corporation Nursery. The improved effectiveness of low inoculum rate by captan may be due to reduction in populations of microorganisms antagonistic to the inoculum. The higher inoculum rate probably compensated for the benefits of captan.

It is interesting to note that midstudy *Pt* indices on seedlings in the four treat-

ments of IMRD inoculum at the Champion-International Corporation Nursery averaged 36 whereas indices on seedlings from the other three nurseries averaged between 72 and 86. The only difference in cultural practice between these nurseries was the use of the systemic fungicide triadimefon at the Champion nursery. It was sprayed on seedlings three times in the spring to control fusiform rust caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* Burdsall & Snow. Recently, Kelley (1982) found that triadimefon inhibited mycelial growth of Pt by 50 percent at a concentration of only 1  $\mu\text{g/ml}$ . Due to its systemic nature, this fungicide may have caused a reduction in susceptibility of short roots to Pt infection. Since no triadimefon-free seedlings that received IMRD inoculum were available for comparison to those that were sprayed, these observations furnish only circumstantial evidence that this fungicide may be involved in the low incidence of Pt ectomycorrhizae in this nursery.

## GENERAL DISCUSSION

Since nursery cultural practices influence ectomycorrhizal development, an attempt was made to correlate these practices with Pt ectomycorrhizal development from IMRD inoculum. IMRD inoculum at 1.08 liter/m<sup>2</sup> was used for these comparisons. It is assumed that cultural practices influence IMRD inoculum and Abbott inocula similarly. Only tests involving loblolly pine seedlings (1-0 stock) were used for these correlations since it was the only tree species grown in enough conventional nurseries to make correlations valid. Final Pt indices of IMRD inocula (without captan), which ranged from 18 to 91, obtained from 20 nursery tests (excluding those at IMRD) carried out in 1977 (7 nurseries), 1978 (8 nurseries), 1979 (3 nurseries), and 1980 (2 nurseries) were used in this analysis. These indices were compared to the following nursery conditions or cultural factors.

<i>Factors</i>	<i>Range of values</i>
Percent short roots ectomycorrhizal with naturally occurring fungi on control seedlings at midstudy assessment	5 to 32
Fumigation effectiveness based on percent reduction of Pythiaceae fungi and nematodes by fumigation	60 to 100
Days of inoculum storage before use	1 to 41
Amount ( $\mu\text{g/g}$ ) of soil nutrients at installation	
Total N	142 to 920
Available P	13 to 54
Exchangeable K	12 to 160
Exchangeable Ca	80 to 2,173
Exchangeable Mg	7 to 164
Percent organic matter at installation	0.4 to 4.6
pH at installation	4.5 to 6.3
Herbicides	
Number of applications	0 to 38
Total rate/ha	0 to 75 and 8,992 l
Fungicides	
Number of applications	0 to 65
Total kg/ha	0 to 163
Insecticides	
Number of applications	0 to 3
Total kg/ha	0 to 56

Soil	
Percent sand	15 to 91
Percent silt	4 to 62
Percent clay	4 to 23
Seedling density/m <sup>2</sup> at study termination	116 to 561

No one of these factors or conditions accounted for more than 13 percent of the variation encountered in final Pt indices. There were not enough of these factors or conditions in common to these nurseries to make comparisons of interactions.

An interesting contrast was found in grams of total plant tissue produced per cm<sup>2</sup> of nursery soil surface between loblolly pine seedlings with a Pt index >50 (IMRD inoculum at 1.08 l/m<sup>2</sup>) and those with natural (control) ectomycorrhizae. Data from 11 conventional nursery tests that (1) grew loblolly pine seedlings, (2) had seedling densities between blocks of the same treatment that did not vary over 60 percent, (3) had a Pt index >50 from the IMRD inoculum treatment, and (4) had mowed seedling tops at least once during the growing season were used in this analysis. The seedling/m<sup>2</sup> parameter was transformed into cm<sup>2</sup> of nursery soil surface per plantable seedling. Total fresh weight was then divided by the latter value and grams of tissue produced per cm<sup>2</sup> of nursery soil surface were obtained. An analysis of variance comparing the two treatments in each of the 11 tests as well as an analysis comparing the 11 IMRD inoculum treatments to the 11 control treatments were made. The results showed that a Pt index >50 increased seedling biomass per unit area of nursery soil in 8 of the 11 nurseries (Table 21). The overall analysis showed that seedlings with a Pt index >50 produced 35 percent more tissue per cm<sup>2</sup> of nursery soil than seedlings with natural ectomycorrhizae. This suggests that the loblolly pine seedlings with a Pt index >50 utilized water and nutrients more effectively than did the routine-grown nursery seedlings. Due to this increased effectiveness, more plantable seedlings per unit area of most nursery soils can be produced with the same cultural practices. An increase in seedling bed density with a corresponding increase in number of plantable seedlings/m<sup>2</sup>, following successful inoculation, can have significant economic impact on nursery production.

With the exception of the 1977 tests at Weyerhaeuser's Arkansas nursery, midstudy assessment of Pt ectomycorrhizal development on loblolly pine seedlings predicted the Pt index at termination of the study. In these nurseries, if the midstudy Pt index was >50 it was also >50 at final assessment. In 6 of the 17 nurseries, Pt indices increased between midstudy and final assessment and in 8 of the 17 there was no significant change. In the other nursery tests in which midstudy Pt indices decreased to significantly lower final Pt indices, no single cultural practice applied to seedlings after midstudy assessment was correlated with this decrease. The amount of nitrogen fertilizer applied to these nurseries after midstudy evaluation ranged from 336 to 448 kg/ha. However, in other nurseries in which Pt indices increased between midstudy and final assessments as much nitrogen fertilizer or more (in one case 784 kg/ha) was applied. Other factors or combinations of factors were likely responsible.

Only one of the four tests installed in Weyerhaeuser's Oklahoma and Arkansas nurseries on loblolly pine seedlings in 1977 and 1978 produced final Pt indices >50 with IMRD inoculum. Most cultural practices used in these nurseries were also common in other nurseries where inoculum was highly effective. The only exception was the use of the herbicide napropamide. Results from tests with loblolly pine at the Oklahoma nursery in 1980 showed that a single application of napropamide did not significantly reduce effectiveness of IMRD inoculum.

TABLE 21. Grams of fresh tissue produced per cm<sup>2</sup> of nursery soil by loblolly pine seedlings with *Pisolithus tinctorius* (Pt) ectomycorrhizae (Pt index > 50) and control seedlings with natural ectomycorrhizae from 11 conventional nursery tests.<sup>1</sup>

Test year and nursery	Seedlings with—	
	Pt index > 50 (g/cm <sup>2</sup> )	Natural ectomycorrhizae (g/cm <sup>2</sup> )
1977		
New Kent, VA	3.9*	3.1
Great Southern, GA	5.8*	3.8
Kimberly-Clark, AL	5.7	5.6
1978		
Great Southern, GA	7.0*	3.1
Westvaco, SC	5.3*	4.3
New Kent, VA	3.1	3.0
1979		
Hiwassee, GA	7.4*	5.9
Champion-International, SC	4.2	3.7
International Paper, MS	4.7*	3.0
1980		
Westvaco, SC	3.3*	1.5
Champion-International, SC	4.5*	3.5
Overall $\bar{X}$	5.0	3.7

<sup>1</sup>\* Denotes significantly different means of treatments within a nursery and also between overall means of the treatments ( $P = 0.05$ ).

Final Pt indices ranged from 34 to 63; both the lowest and highest Pt indices occurred in the napropamide treatment (Marx, unpublished data). Obviously, factors other than the use of napropamide are responsible for inoculum ineffectiveness in these nurseries.

In comparison of results from all studies with loblolly pine seedlings, cultural practices such as previous cover or seedling crops, type and amounts of organic amendments added to the nursery soil before installation of the study, effectiveness of soil fumigation, and types or amounts of mulch and seed treatment, could not be correlated with inoculum effectiveness.

The lack of correlation of effectiveness of soil fumigation with effectiveness of inoculum in the loblolly pine tests does not indicate a lack of importance of fumigation (Marx and others 1978). It may mean that no fumigation was done so improperly as to negate its effect. Test results in 1978 at the IMRD Microplot Nursery on loblolly pine and northern red oak where fumigation was intentionally done very poorly showed the significance of fumigation to inoculum effectiveness. Soil fumigation in the fall preceding soil inoculation the next spring also was not as advantageous to inoculum effectiveness as spring fumigation. In 1978, only 30 percent (3 of 10) of the nurseries that fumigated soil in the fall had midstudy Pt indices > 50 from IMRD inoculum whereas 80 percent (8 of 10) of those that fumigated soil in the spring did. This suggests that the several months of soil exposure between fall fumigation and spring inoculation allows colonization of soil by saprophytic organisms and other ectomycorrhizal fungi which may serve as competitors and possible antagonists to introduced Pt inocula.

Because the growth of loblolly pine seedlings varied considerably among nurseries (6.1 to 50.9 g total fresh weights), there was no valid way of correlating amounts of Pt ectomycorrhizae and seedling growth. The same problem was

encountered in attempts to correlate amounts of *Pt* ectomycorrhizae and percent of seedlings culled, which varied from 6 to 70 percent among nurseries. The different cultural practices and other factors in these nurseries so strongly influenced seedling growth that the influence of *Pt* ectomycorrhizae was masked.

A comparison of all inoculum treatment combinations (regardless of inoculum source) on 1-0 pine seedlings in conventional nurseries producing final *Pt* indices >50 revealed some interesting points. A total of 69 inoculum treatment combinations produced *Pt* indices >50; 24 of these (35 percent) resulted in larger seedlings (total fresh weight) and 22 of these (32 percent) had significantly fewer culls than control seedlings. In those inoculum treatment combinations in which seedling growth response was not significant, lack of growth differences was not because of deficiencies of ectomycorrhizae. Control seedlings in these tests had an average of 31 percent short roots ectomycorrhizal with naturally occurring fungi. In those treatments in which cull percentage was not affected by *Pt* ectomycorrhizae, the control seedlings averaged less than 27 percent culls. It seems that seedlings growing well in certain nurseries with abundant naturally occurring ectomycorrhizae are not stimulated by additional ectomycorrhizae formed by *Pt*. Also, if the cull rate is low, then additional *Pt* ectomycorrhizae may not significantly reduce this amount. However, in several of these tests where treatment differences were not significant, an erratic seedling density due to uncontrollable factors (rain, reseeding, poor seed germination, etc.) could have confounded the effect of treatment. The practice of mowing seedling tops during the growing season in certain southern nurseries also removes significant biomass from final measurements. This cultural practice, which allows the smaller seedlings to catch up with the faster growing seedlings that had their tops mowed, decreases the dominant effect of larger seedlings. Top pruning in September and October should also significantly decrease seedling cull percentages in southern pine nurseries.

Results from the 2-0 nursery tests and the one 3-0 nursery test in the more northern and western parts of the United States revealed poor development of *Pt* ectomycorrhizae. After the first growing season, only 7 of the twelve 2-0 nursery tests had midstudy *Pt* indices >50 and only 2 had final *Pt* indices >50. Inoculation with either IMRD or Abbott inoculum in four 2-0 tests stimulated seedling growth and reduced culls; in three of these tests either midstudy or final *Pt* indices >50 were correlated with these significant differences. No test on Douglas-fir seedlings produced midstudy or final *Pt* indices >50 nor was seedling growth affected by soil inoculation. In the earlier container-grown seedling tests (Marx and others 1982), both IMRD and Abbott inocula formed high *Pt* indices on Douglas-fir seedlings in Oregon with a rooting medium of a 1:1 volume ratio of vermiculite and peat moss. These conflicting results suggest that an inoculum formulation that is effective in the near-sterile conditions used in the production of container-grown seedlings is not necessarily effective on the same tree species in bare-root nurseries where physical, chemical, and biological conditions in soil are quite different. The possible significance of soil fumigation in the fall in these negative nursery tests has been discussed.

Since there were insufficient numbers of 2-0 nurseries with common tree species, correlation of *Pt* indices with various cultural practices was not attempted. However, in nearly all of these 2-0 tests, initial soil fertility was generally much higher than that of 1-0 tests in more southern areas. *Pt* ectomycorrhizae persisted well in the USDA-SCS Nursery for 2 years indicating that, in certain northern nurseries, its supplantation by other fungi is slow. Generally, however, in the 2-0 nurseries and the one 3-0 nursery test, a high *Pt* index after the first growing season was followed by a progressively lower and often unacceptable *Pt* index after the second growing season. By the end of the third growing season in the 3-0 tests, only a small amount of *Pt* ectomycorrhizae was present.

Since only moderate amounts of fertilizer were added to most 2-0 and 3-0 nurseries during the second or third growing season, it is doubtful that fertilizers played a key role in supplantation of Pt. In these northern pine nurseries, severe winter conditions may have killed some of the ectomycorrhizae and short roots formed during the previous growing season. This would mean that ectomycorrhizae formed the next growing season came from surviving fungal propagules which could infect newly formed roots. It may be that some ectomycorrhizae persist for only 1 year on red pine in certain of these northern nurseries and if winter survival of propagules of the southern strain of Pt is low, then its ectomycorrhizal development the following growing season will be less than that of previous growing seasons. This conjecture is supported by the fact that Pt indices obtained after the first growing season (before winter) in the northern nurseries were as high or, in some tests, even higher than those obtained in the southern nurseries. This indicates that nursery growing conditions (i.e., spring fumigation and moderate fertility) during the first season were adequate for Pt ectomycorrhizae to develop but changes occurred during the winter. Perhaps use of more cold-tolerant strains of Pt or reinoculation of these seedlings in the spring with Pt spores would decrease supplantation of Pt by other fungi and increase the likelihood of obtaining seedlings with Pt indices >50 for subsequent field tests.

The production of fruit bodies of Pt was strongly correlated with successful establishment of Pt ectomycorrhizae on seedling roots, especially in the southern nurseries. Size of Pt fruit bodies varied considerably as did the stalked or non-stalked trait. Neither of these traits is valid in describing Pt since a single isolate produces such a wide variance in these traits. Basidiospores from these fruit bodies can function as a secondary source of inoculum to reinoculate nursery soils of the current crop. This reinoculation should result in a higher incidence of Pt ectomycorrhizae on seedlings than reported here, because in most of these tests, fruit bodies of Pt were immediately removed from plots to minimize contamination of other treatments.

Tt was the most frequently encountered naturally occurring ectomycorrhizal fungus in these tests. Fruit bodies of Tt were found on all tree species in 41 of the 45 conventional nursery tests in 23 of the 25 states. Fruit bodies of *R. nigrescens* were found only in the Georgia and Florida nurseries. Ectomycorrhizae formed by *Cenococcum geophilum* were detected in Michigan and South Carolina nurseries, and fruit bodies of *Laccaria laccata* were found only in the Washington nursery. The widespread occurrence of Tt in nurseries supports earlier reports (Marx 1980) that it is the major ectomycorrhizal fungus on roots of nursery seedlings used for forestation programs in the United States.

## CONCLUSIONS

The results of these tests on bare-root seedlings clearly show that viable vegetative inoculum of Pt in a substrate of vermiculite-peat moss-nutrient can be produced with industrial fermentation equipment and production procedures for practical use in forestry. The vegetative inoculum of Pt produced by Abbott Laboratories has the trademark MycoRhiz®.<sup>4</sup> Although early formulations of Pt inoculum produced by Abbott Laboratories lacked consistent effectiveness, the 1980 formulations produced abundant ectomycorrhizae on root systems of pine resulting in Pt indices >50. As discussed earlier, results from outplanting studies (Marx and others 1977a, Kais and others 1981, Ruehle and others 1981, Ruehle and

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<sup>4</sup> Registered trademark Abbott Laboratories, North Chicago, IL, for vegetative inoculum of *Pisolithus tinctorius*.

Brendemuehl 1981) showed that an index of 50 is the minimum level of root colonization by Pt required to significantly increase survival and growth of southern pine seedlings on reforestation sites. Additional research is needed to determine the minimum Pt index required for other tree species and for southern pines planted on adverse sites.

The major differences between the 1980 formulations of Abbott inoculum and those produced earlier were that the latter excluded peat moss (1979), did not leach inoculum before drying (1978), or excluded both factors (1977). Both factors were proven to be important in the effectiveness of the 1980 formulation of Abbott inoculum. As discussed earlier (Marx and others 1982), MycoRhiz<sup>®</sup> formulations with final pH values greater than 6.0 were not very effective in forming Pt ectomycorrhizae on loblolly pine seedlings. Inoculum with either 5 or 10 percent peat moss as a component of the initial vermiculite-nutrient substrate in the fermentor stabilized pH between 5.0 and 5.6. Acidification of pure vermiculite-nutrient inoculum with various buffers or peat moss after fermentation was not successful. The use of peat moss as an acidifying agent for vermiculite was originally developed because of failures in the permanent acidification of vermiculite with routine chemical buffers (Marx and Zak 1965). The acidophilic nature of Pt and the moderately acidic condition of nursery soils suggest that effective inoculum should also be moderately acid.

Unfortunately, no single inoculum characteristic determined before nursery testing was consistently correlated with inoculum effectiveness. However, results of the bare-root seedling tests, the fast assay technique, and the container tests (Marx and others 1982) showed that the most effective inoculum had (1) abundant hyphae of Pt inside the vermiculite particles, (2) pH between 4.5 and 6.0, (3) low amount of microbial contaminants, and (4) low amounts of residual glucose as a consequence of leaching the inoculum before drying. Undoubtedly, leaching removes many other nutrients as well. Results of the various tests using inoculum rates between 1.62 and 0.27 l/m<sup>2</sup> of soil surface indicate that 1.08 liters of inoculum/m<sup>2</sup> is the most effective.

MycoRhiz<sup>®</sup> can be effective in forming abundant Pt ectomycorrhizae on both container-grown (Marx and others 1982) and bare-root seedlings of various tree species. However, as discussed earlier, there are still undefined factors or combinations of factors in certain bare-root nurseries which preclude consistent inoculum effectiveness, and, therefore, further study is required. However, since Pt is currently the only ectomycorrhizal fungus for which the technology exists to introduce it into nurseries on a large scale, we suggest that work be undertaken to produce seedlings with Pt indices >50. Only then can the next phase of this program, outplanting studies, be undertaken to ascertain the significance of this specific ectomycorrhizal association to survival and growth of tree seedlings. Major benefits of this or any other ectomycorrhizal fungus to forestry are aimed not only at seedlings production programs but ultimately at improving artificial regeneration or disturbed-sites reclamation programs. Since this work was completed, Walker and others (1982) reported that Virginia pine seedlings with Pt indices >50 formed in the 1978 Vallonia, Indiana, nursery test with Abbott and IMRD inocula grew faster and exhibited less moisture stress on a coal spoil in Tennessee than seedlings with natural ectomycorrhizae or with a Pt index <50.

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TABLE 22. *Index of experiments by tree species, nursery, and year.*

Species and nursery	Year	Page
Austrian pine		
Bessey Tree Nursery, NE	1978	77
Oklahoma State Nursery, OK	1978	74
Douglas-fir		
Coeur d'Alene Nursery, ID	1978	71
Industrial Forestry Association Nursery, WA	1978	76
Tyee Tree Farm Nursery, OR	1978	70
Eastern white pine		
Edwards State Nursery, NC	1978	77
Marietta State Nursery, OH	1978	73
Parsons State Nursery, WV	1978	72
Loblolly pine		
Champion-International Corporation Nursery, SC	1979, 1980	98, 120
Great Southern Paper Company Nursery, GA	1977, 1978	35, 53
Hiwassee Land Company Nursery, GA	1979	99
IMRD Microplot Nursery, GA	1977, 1978, 1979, 1980	43, 53, 91, 111
International Paper Company Nursery, MS	1979	95
Kimberly-Clark Corporation Nursery, AL	1977, 1978	37, 63
New Kent Nursery, VA	1977, 1978	39, 75
Union State Nursery, IL	1978	68
Waynesboro Nursery, MS	1978	65
Westvaco Corporation Nursery, SC	1977, 1978, 1980	36, 55, 123
Weyerhaeuser Company, AR	1977, 1978	33, 65
Weyerhaeuser Company Nursery, OK	1977, 1978	32, 64
Longleaf pine		
Beauregard Nursery, LA	1977, 1978	31, 67
Griffith Experimental Nursery, NC	1977	41
Northern red oak		
IMRD Microplot Nursery, GA	1977	43
Ponderosa pine		
Bessey Tree Nursery, NE	1977	38
Placerville Nursery, CA	1977	38
Red pine		
NEPCO Lake Nekoosa-Edwards Corporation Nursery, WI	1978	79
Potlatch Nursery, MN	1978	78
J. W. Toumey Nursery, MI	1978	81
USDA-SCS Nursery, MI	1978	80
Sand pine		
Andrews Nursery, FL	1977	25
Shortleaf pine		
W. W. Ashe Nursery, MS	1977	23
Kentucky Dam Nursery, KY	1977	42
George O. White Nursery, MO	1978	69
Slash pine		
Buckeye Cellulose Corporation Nursery, FL	1977, 1978, 1979, 1980	34, 66, 92, 116
Virginia pine		
Griffith Experimental Nursery, NC	1977	41
Pinson Nursery, TN	1977	40
Vallonia State Nursery, IN	1978, 1979	70, 100

APPENDIX I. Cropping history, fertilizers and fumigants used prior to installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1977.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants			
	Type	kg/ha	Percent composition <sup>2</sup>		kg/ha	Date
Ashe, MS: Shortleaf pine						
Pine seedlings in 1974, 1975 and 1976	13-13-13	56	MyBr	98		April
	Trace ele- ments	34	Chloro	2	487	1977
			Inert	0		
Andrews, FL: Sand pine						
Grain sorghum in 1974 and 1976 and pine seedlings in 1975	10-10-10	224	MyBr	67		March
	Super PO <sub>4</sub>	280	Chloro	33	515	1977
	K <sub>2</sub> SO <sub>4</sub>	336	Inert	0		
Beauregard, LA: Longleaf pine						
Pine seedlings in 1974 and 1975; 5-cm layer of pine bark disked in, then grain sorghum in 1976	lime	560	MyBr	98		March
	KCl	336	Chloro	2	392	1977
	CaSO <sub>4</sub>	336	Inert	0		
	Super PO <sub>4</sub>	224				
Weyerhaeuser, OK (old area): Loblolly pine						
Soil shaping in 1974 and pine seedlings in 1975 and 1976	10-20-10	560	MyBr	98		April
	lime	1,120	Chloro	2	358	1977
			Inert	0		
Weyerhaeuser, OK (new area): Loblolly pine						
In pasture, then soil shaping in 1976; 334 m <sup>3</sup> /ha of sawdust disked in July 1976	14-42-0	800	MyBr	98		April
	lime	1,120	Chloro	2	358	1977
			Inert	0		
Weyerhaeuser, AR: Loblolly pine						
Pine seedlings in 1974 and rye grass in 1975 and 1976; 1.5-cm layer of saw- dust disked in August 1976	NH <sub>4</sub> NO <sub>3</sub>	775	MyBr	98		March
	10-20-20	560	Chloro	2	358	1977
	lime	1,120	Inert	0		
Buckeye, FL: Slash pine						
Pine seedlings in 1974, rye grass (win- ter) and millet (summer) in 1975 and 1976	5-10-15	784	MyBr	68.4		March
			Chloro	1.6	672	1977
			Inert	30.0		
Great Southern, GA: Loblolly pine						
Peanuts in 1974, corn in 1975 and mil- let (summer) and rye grass (winter) in 1976; 2.5-cm layer of pine bark disked in September 1976	NH <sub>4</sub> NO <sub>3</sub>	168	MyBr	98		April
			Chloro	2	403	1977
			Inert	0		
Westvaco, SC: Loblolly pine						
Pine seedlings in 1974, corn (summer) and rye grass (winter) in 1975 and su- dan grass in 1976	10-10-10	897	MyBr	98		April
			Chloro	2	398	1977
			Inert	0		
Kimberly-Clark, AL: Loblolly pine						
Pine seedlings in 1974 and 1976, corn in 1975; 1.5-cm layer of pine bark disked in April 1977	10-10-10	672	MyBr	98		April
			Chloro	2	672	1977
			Inert	0		
Placerville, CA: Ponderosa pine						
Pine seedlings in 1974 and 1975, crown vetch in 1976	16-20-0	180	MyBr	75		April
			Chloro	25	448	1977
			Inert	0		

## APPENDIX I. Continued.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants		
	Type	kg/ha	Percent composition <sup>2</sup>	kg/ha	Date
Bessey, NE: Ponderosa pine					
Juniper seedlings till 1976 then sudan grass from May to July 1976 and oats till September 1976	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	336	MyBr	68.4	May 1977
			Chloro	1.6	
			Inert	30.0	
New Kent, VA: Loblolly pine					
Pine seedlings in 1974 and 1975; 2.5-cm layer of sawdust disked in then sudan grass in 1976; area subsoiled to 45 cm in March 1977	lime	2,240	MyBr	98	April 1977
	K <sub>2</sub> O	157	Chloro	2	
			Inert	0	
Pinson, TN: Virginia pine					
Sudan grass in 1974 and 1975; pine seedlings in 1976	13-13-13	620	MyBr	98	March 1977
			Chloro	2	
			Inert	0	
Griffith, NC: Virginia and loblolly pines					
Peas in 1974 and fallow in 1975 and 1976	8-8-8	560	MyBr	68.6	April 1977
			Chloro	1.4	
			Inert	30.0	
Kentucky Dam, KY: Shortleaf pine					
Pine seedlings in 1974 and 1976; peas in 1975	15-15-15	336	MyBr	98	April 1977
			Chloro	2	
			Inert	0	

<sup>1</sup> Fertilizers were applied just before bed shaping and study installation.<sup>2</sup> MyBr—methyl bromide; Chloro—chloropicrin.

APPENDIX II. Chemical ( $\mu\text{g/g}$ ) and physical characteristics of soil at installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1977.

Nursery location and tree species	Total N	Avail- able P	Exchangeable			Percent organic matter	pH	Percent sand	Percent silt	Percent clay	Soil type
			K	Ca	Mg						
Ashe, MS: Shortleaf pine	308	30	81	152	23	1.1	4.3	81	14	5	loamy sand
Andrews, FL: Sand pine	196	126	13	404	6	0.8	4.8	94	3	3	sand
Beauregard, LA: Longleaf pine	256	27	104	332	41	1.4	5.2	79	16	15	loamy sand
Weyerhaeuser, OK, (old area): Loblolly pine	187	42	51	89	5	0.5	5.2	89	7	4	sand
Weyerhaeuser, OK, (new area): Loblolly pine	142	43	31	106	12	0.5	5.5	90	6	4	sand
Weyerhaeuser, AR: Loblolly pine	283	21	41	261	7	1.3	5.3	87	8	5	sand
Buckeye, FL: Slash pine	261	53	26	139	15	1.2	5.2	95	3	2	sand
Great Southern, GA: Loblolly pine	268	25	42	226	19	1.1	5.1	89	7	4	sand
Westvaco, SC: Loblolly pine	920	53	132	419	109	4.6	4.5	83	10	7	loamy sand
Kimberly-Clark, AL: Loblolly pine	780	54	160	2,173	130	3.8	5.1	53	30	17	sandy loam
Placerville, CA: Ponderosa pine	1,240	19	240	2,007	113	3.8	5.4	53	27	20	sandy loam
Bessey, NE: Ponderosa pine	367	58	61	1,273	66	0.9	5.6	90	5	5	sand
New Kent, VA: Loblolly pine	544	49	136	221	79	2.4	4.8	89	7	4	sand
Pinson, TN: Virginia pine	660	33	89	1,281	78	1.4	5.2	42	45	13	loam
Griffith, NC: Virginia pine	672	104	95	276	24	1.9	4.2	85	9	6	loamy sand
Griffith, NC: Longleaf pine	654	155	97	398	26	1.8	4.4	84	10	6	loamy sand
Kentucky Dam, KY: Shortleaf pine	523	55	110	341	26	1.1	4.8	66	24	10	sandy loam
IMRD, GA:											
Loblolly pine	318	19	49	190	27	2.3	5.1	83	7	10	loamy sand
Northern red oak	320	20	47	570	27	2.3	5.9	83	7	10	loamy sand

APPENDIX III. Fertilizers and pesticides added during nursery tests of vegetative inoculum of *Pisolithus tinctorius* begun in 1977.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
Ashe, MS: Shortleaf pine											
NH <sub>4</sub> NO <sub>3</sub>	13	7	trifluralin	1.1 kg	1	ferbam	2.2	23	diazinon	1.1	3
			EPTC	3.4 kg	1	captan	1.7	3			
			nitrofen	3.4 kg	8						
Andrews, FL: Sand pine											
10-10-10	224	1	mineral spirits	187 l	28	ferbam	2.2	12	malathion	2.2	1
K <sub>2</sub> SO <sub>4</sub>	280	1	bifenox	14 l	3						
NH <sub>4</sub> NO <sub>3</sub>	140	3	nitrofen	3.4 kg	2						
Beauregard, LA: Longleaf pine											
Fe chelate	3.4	2	None used			captan	6.7	2	None used		
Super PO <sub>4</sub>	224	1		PCNB	6.7	1					
NH <sub>4</sub> NO <sub>3</sub>	224	1		Banrot	2.2	1					
				ferbam	2.2	11					
Weyerhaeuser, OK (old and new areas): Loblolly pine											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	112	3	napropamide	1.1 kg	1	ferbam	2.2	5	disulfoton	9	3
KCl	112	3	mineral spirits	187 l	4						
			bifenox	3.9 kg	4						
Weyerhaeuser, AR: Loblolly pine											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	112	4	napropamide	1.1 kg	2	captan	13.4	1	disulfoton	6.7	1
			bifenox	3.9 kg	6	ferbam	2.2	15			
			prometryne	1.1 kg	1						
			trifluralin	2.3 l	1						
			mineral spirits	187 l	3						
Buckeye, FL: Slash pine											
NH <sub>4</sub> NO <sub>3</sub>	17	3	diphenamid	4.5 kg	1	ferbam	3.4	44	dicofol	6.7	1
KCl	35	1	prometryne	1.2 kg	1						
			mineral spirits	140 l	5						

APPENDIX III. Continued.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
Great Southern, GA: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	90	1	diphenamid	9 kg	1	ferbam	2.5	55	malathion	2.2	1
17-17-17	112	1	nitrofen	4.5 l	34	chlorothalonil	3.4	1			
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	134	2				PCNB; captan	1.1	1			
KCl	112	1									
Westvaco, SC: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	112	4	nitrofen	9 l	5	ferbam	2.2	33	chlordane	28	1
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	112	1	mineral spirits	281 l	32						
KCl	112	1	diphenamid	4.5 kg	1						
Kimberly-Clark, AL: Loblolly pine											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	224	2	diphenamid	6.7 kg	1	ferbam	2.8	5			
15-10-15	168	1	prometryne	1.7 kg	2				None used		
			mineral spirits	276 l	4						
Bessey, NE: Ponderosa pine											
<i>First season</i>											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	43	1	diphenamid	5.6 kg	3	None used			None used		
<i>Second season</i>											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	112	2	diphenamid	5.6 kg	1	None used			dimethoate	2.2	1
New Kent, VA: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	112	5	mineral spirits	310 l	10	None used			None used		
Pinson, TN: Virginia pine											
urea	112	1	mineral spirits	280 l	1	captan	4.5	1	malathion	2.2	2
NaNO <sub>3</sub>	896										
Griffith, NC: Virginia and loblolly pines											
NH <sub>4</sub> NO <sub>3</sub>	28	4	None used			None used			None used		

## APPENDIX III. Continued.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
Kentucky Dam KY: Shortleaf pine											
NH <sub>4</sub> NO <sub>3</sub>	112	2	bifenox	3.4 kg	1	captan	4.5	2	None used		
			trifluralin	1.1 kg	2						
IMRD, GA: Loblolly pine and northern red oak											
NH <sub>4</sub> NO <sub>3</sub>	168	2	None used			None used			dimethoate	3.0	12

APPENDIX IV. Cropping history, fertilizers and fumigants used prior to installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1978.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants			Date
	Type	kg/ha	Percent composition <sup>2</sup>		kg/ha	
Great Southern, GA: Loblolly pine						
Corn in 1975, millet in 1976 and rye grass in 1977; 2.5-cm layer of pine bark disked in August 1977	10-10-10	560	MyBr	98	490	March 1978
			Chloro	2		
			Inert	0		
Westvaco, SC: Loblolly pine						
Sorghum in 1975, pine seedlings in 1976, corn (summer) and rye grass (winter) in 1977	10-10-10	896	MyBr	98	400	April 1978
			Chloro	2		
			Inert	0		
Kimberly-Clark, AL: Loblolly pine						
Corn in 1975 and 1977, pine seedlings in 1976; 2.5-cm layer of pine bark disked in March 1978	0-14-14	840	MyBr	67	448	April 1978
	10-10-10	672	Chloro	33		
			Inert	0		
Weyerhaeuser, OK: Loblolly pine						
Pasture in 1975, soil shaping in 1976, oats (summer) and rye grass (winter) in 1977	lime	1,120	MyBr	98	358	April 1978
	10-20-10	560	Chloro	2		
			Inert	0		
Weyerhaeuser, AR: Loblolly pine						
Pine seedlings in 1975 and 1977, peas in 1976	MgSO <sub>4</sub>	112	MyBr	98	358	March 1978
	10-20-10	336	Chloro	2		
			Inert	0		
Waynesboro, MS: Loblolly pine						
Pine seedlings in 1975 and 1976, peas in 1977	K <sub>2</sub> O	173	MyBr	98	510	March 1978
	NH <sub>4</sub> NO <sub>3</sub>	116	Chloro	2		
			Inert	0		
Buckeye, FL: Slash pine						
Millet (summer) and rye grass (winter) in 1975 and 1977, pine seedlings till December then rye grass till February 1978	5-10-25	784	MyBr	68.4	549	March 1978
	+ trace		Chloro	1.6		
	elements		Inert	0		
Beauregard, LA: Longleaf pine						
Rye grass seedlings in 1975 and 1976, sudan grass in 1977	lime	1,680	MyBr	98	400	March 1978
	21 K <sub>2</sub> O:		Chloro	2		
	25 MgSO <sub>4</sub>	448	Inert	0		
Union State, IL: Loblolly pine						
Pine seedlings in 1975 and 1976, fallow in 1977	6-24-24	672	MyBr	98	672	October 1977
			Chloro	2		
			Inert	0		
George O. White, MO: Shortleaf pine						
Sorghum-sudan grass in 1975 and 1977, pine seedlings in 1976	8-24-40	560	MyBr	98	490 (surface appl.)	August 1977
			Chloro	2		
			Inert	0		
Vallonia, IN: Virginia pine						
Hardwood seedlings in 1975 and 1976, sorghum-sudan grass (summer) and rye grass (fall) in 1977	12-12-12	448	MyBr	98	448	May 1978
			Chloro	2		
			Inert	0		

## APPENDIX IV. Continued.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants			Date
	Type	kg/ha	Percent composition <sup>2</sup>		kg/ha	
Tyee Tree Farm, OR: Douglas-fir						
Pasture since 1975, soil shaping in 1977	0-20-0	168	MyBr	67	431	November 1977
			Chloro	33		
			Inert	0		
Coeur d'Alene, ID: Douglas-fir						
Peas in 1975 and 1976, oats in 1977	None used		MyBr	67	392	August 1977
			Chloro	33		
			Inert	0		
Parsons, WV: Eastern white pine						
Rye grass since 1975	lime	1,124	MyBr	98	487	September 1977
			Chloro	2		
			Inert	0		
Marietta, OH: Eastern white pine						
Sudan grass (summer) and wheat (winter) in 1975 and 1977, pine seedlings in 1976	12-12-12	536	MyBr	98	392	October 1977
			Chloro	2		
			Inert	0		
Oklahoma State, OK: Austrian pine						
Fallow in 1975 and hardwood seedlings in 1976 and 1977	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	190	MyBr	98	1,066 (surface appl.)	April 1978
	granular S	560	Chloro	2		
	milorganite		Inert	0		
	(6-2-0)	1,120				
New Kent, VA: Loblolly pine						
Sudan grass in 1975 and pine seedlings in 1976 and 1977; 2.5-cm layer of partially decayed sawdust disked in February 1978	lime KCl	2,240 261	MyBr	98	356	March 1978
			Chloro	2		
			Inert	0		
Industrial Forestry Assoc., WA: Douglas-fir						
Douglas-fir in 1975 and 1976, fallow in 1977	10-20-20 0-45-0	392 224	MyBr	67	392	October 1977
			Chloro	33		
			Inert	0		
Bessey, NE: Austrian pine						
Pine seedlings in 1975 and 1976, sudan grass (summer) and oats (winter) in 1977	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	168	MyBr	68.4	560	May 1978
			Chloro	1.6		
			Inert	30.0		
Edwards, NC: Eastern white pine						
Pine seedlings in 1976 and fallow in 1975 and 1977	10-10-10 lime	392 2,240	MyBr	68.4	392	October 1977
			Chloro	1.6		
			Inert	30.0		
Potlatch, MN: Red pine						
Pine seedlings in 1975 and 1976; 4-cm layer of peat moss disked in May 1978	12-12-12	112	MyBr	98	492	June 1978
			Chloro	2		
			Inert	0		
Nepco Lake, WI: Red pine						
Pine seedlings in 1975 and 1976, sudan grass in 1977	NH <sub>4</sub> NO <sub>3</sub>	336	MyBr	98	700 (surface appl.)	May 1978
	21 K <sub>2</sub> O:		Chloro	2		
	25 MgSO <sub>4</sub>	280	Inert	0		
	super PO <sub>4</sub>	224				

# APPENDIX IV. Continued.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants		
	Type	kg/ha	Percent composition <sup>2</sup>	kg/ha	Date
USDA-SCS, MI: Red pine					
Crown vetch in 1975 and sorghum-sudan grass in 1976 and 1977	None used		MyBr	98	October 1977
			Chloro	2	
			Inert	0	
Toumey, MI: Red pine					
Rye grass in 1976 and 1977, pine seedlings in 1975; 4-cm layer of peat moss disked in August 1977	0-0-50	336	MyBr	67	October 1977
	20-0-20	560	Chloro	33	
	lime	1,120	Inert	0	

<sup>1</sup> Fertilizers were applied just before bed shaping and study installation.

<sup>2</sup> MyBr—methyl bromide; Chloro—chloropicrin.

APPENDIX V. Chemical ( $\mu\text{g/g}$ ) and physical characteristics of soil at installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1978.

Nursery location and tree species	Total N	Avail- able P	Exchangeable			pH	Per- cent sand	Per- cent silt	Per- cent clay	Soil type
			K	Ca	Mg					
IMRD, GA: Loblolly pine	321	21	46	184	24	5.2	84	6	10	loamy sand
Great Southern, GA: Loblolly pine	504	32	61	295	28	4.8	83	7	10	loamy sand
Westvaco, SC: Loblolly pine	282	27	41	80	27	4.7	86	8	6	loamy sand
Kimberly-Clark, AL: Loblolly pine	731	25	119	427	49	4.5	48	34	18	loam
Weyerhaeuser, OK: Loblolly pine	242	30	67	103	11	4.7	91	4	5	sand
Weyerhaeuser, AR: Loblolly pine	510	28	59	226	19	4.9	83	10	7	loamy sand
Waynesboro, MS: Loblolly pine	731	27	159	157	53	4.5	65	22	13	sandy loam
Buckeye, FL: Slash pine	237	64	59	101	21	5.0	94	3	3	sand
Beauregard, LA: Longleaf pine	550	63	87	424	58	5.3	69	21	10	sandy loam
Union State, IL: Loblolly pine	845	48	127	719	106	5.0	38	52	10	silt loam
George White, MO: Shortleaf pine	788	51	115	717	149	5.4	40	43	17	loam
Vallonia, IN: Virginia pine	409	113	63	105	8	4.3	82	10	8	loamy sand
Tyce, OR: Douglas-fir	1,643	43	154	117	291	5.0	75	12	13	sandy loam
Coeur d'Alene, ID: Douglas-fir	540	48	95	718	75	4.9	75	16	9	sandy loam
Parsons State, WV: Eastern white pine	1,184	29	78	745	19	5.2	70	17	13	sandy loam
Marietta State, OH: Eastern white pine	1,134	91	108	408	84	4.8	85	8	7	loamy sand
Oklahoma State, OK: Austrian pine	777	32	130	629	246	5.6	47	42	11	loam
New Kent, VA: Loblolly pine	566	34	34	261	85	5.3	88	8	4	sand
Indust. For. Assoc., WA: Douglas-fir	1,222	82	105	1,256	220	4.8	55	35	10	sandy loam
Bessey, NE: Austrian pine	474	38	49	467	40	4.9	91	4	5	sand
Edwards, NC: Eastern white pine	464	24	62	161	42	4.4	67	23	11	sandy loam
Potlatch, MN: Red pine	1,732	37	54	747	90	4.6	67	26	7	sandy loam
NEPCO, WI: Red pine	1,637	38	18	219	54	4.2	88	6	6	loamy sand
USDA-SCS, MI: Red pine	658	28	30	114	23	4.8	88	7	5	loamy sand
Toumey, MI: Red pine	1,156	25	74	96	17	4.7	86	9	5	loamy sand

APPENDIX VI. Fertilizers and pesticides added during nursery tests of vegetative inoculum of *Pisolithus tinctorius* begun in 1978.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
IMRD, GA: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	168	3	None used			None used			dimethoate	3.0	10
Great Southern, GA: Loblolly pine											
10-10-10	168	1	nitrofen	2.2 kg	34	ferbam	2.5	60	malathion	1.1	1
NH <sub>4</sub> NO <sub>3</sub>	67	1	bifenox	2.8 kg	1	benomyl	1.1	1			
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	134	2				chlorothalonil	2.2	4			
KCl	112	1									
Westvaco, SC: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	112	4	bifenox	2.8 kg	3	ferbam	2.2	52	chlordane (10%)	56	1
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	112	1	nitrofen	9.3 l							
KCl	112	1									
Kimberly-Clark, AL: Loblolly pine											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	224	2	mineral spirits	187 l	2	ferbam	2.8	1	chlordane (Technical grade)	3.4	1
Weyerhaeuser, OK: Loblolly pine											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	112	7	nitrofen	4.5 l	7	ferbam	2.2	6	disulfoton	11	2
			bifenox	4.2 kg	1						
			napropamide	1.1 kg	1						
Weyerhaeuser, AR: Loblolly pine											
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	112	3	bifenox	3.9 kg	3	ferbam	2.2	15	None used		
KCL	112	2	napropamide	1.1 kg	1						
Waynesboro, MS: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	112	1	bifenox	2.2 kg	3	ferbam	2.2	17	None used		

## APPENDIX VI. Continued.

[illegible]

APPENDIX VI. Continued.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
Coeur d'Alene, ID: Douglas-fir											
First season											
21-0-0	140	2	diphenamid	7.0 kg	4	None used			None used		
			DCPA	15.7kg	4						
Second season											
16-20-0	289	1	diphenamid	7.0 kg	2	None used			None used		
21-0-0	224	2	DCPA	15.7 kg	2						
Parsons, WV: Eastern white pine											
First season											
NH <sub>4</sub> NO <sub>3</sub>	504	6	None used			None used			None used		
0-20-20	504	6									
5-10-10	540	1									
Second season											
NH <sub>4</sub> NO <sub>3</sub>	504	2	None used			None used			None used		
0-20-20	504	2									
Marietta, OH: Eastern white pine											
First season											
12-12-12	280	2	diphenamid	4.5 kg	1	None used			None used		
Second season											
12-12-12	224	3	diphenamid	4.5 kg	1						
Oklahoma State, OK: Austrian pine											
First season											
18-46-0	112	3	bifenox	3.4 kg	1	benomyl	16.8	1	None used		
15-15-4	112	1				copper salt	1.0	1			
21-0-0	196	1									
Second season											
	None used		bifenox	3.4 kg	3	None used			dimethoate	0.6	4

## APPENDIX VI. Continued.

[illegible]

APPENDIX VI. Continued.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
<i>Second season</i>											
16-16-16	224	1	diphenamid	11.2 kg	1	maneb	2.2	8	malathion	1.1	10
MgSO <sub>4</sub>	22	4							carbaryl	2.2	7
NH <sub>4</sub> NO <sub>3</sub>	112	4									
Toumey, MI: Red pine											
<i>First season</i>											
milorganite			DCPA	6.7 kg	3	maneb	3.4	3	diazinon	4.5	2
(6-2-0)	420	2				chlorothalonil	2.2	5			
<i>Second season</i>											
NH <sub>4</sub> NO <sub>3</sub>	112	1	DCPA	6.7 kg	2	maneb	3.4	2	malathion	1.1	1
						chlorothalonil	2.2	9	diazinon	4.5	1
<i>Third season</i>											
NH <sub>4</sub> NO <sub>3</sub>	112	1	DCPA	6.7	1	maneb	3.4	4	malathion	1.1	2
21-0-0	84	1				chlorothalonil	2.2	10			

APPENDIX VII. Cropping history, fertilizers and fumigants used prior to installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1979.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants			Date
	Type	kg/ha	Percent composition <sup>2</sup>		kg/ha	
Buckeye, FL: Slash pine						
Millet (summer) and rye grass (winter) of 1976 and 1978, pine seedlings in 1977	10-10-10	784	MyBr	68.4		March 1979
			Chloro	1.6	550	
			Inert	30.0		
International Paper, MS: Loblolly pine						
Soybeans in 1976, fallow in 1977 and peas in 1978; 5-cm layer of hardwood bark disked in August 1977	NH <sub>4</sub> NO <sub>3</sub> 6-12-12	336	MyBr	98		October 1978
		448	Chloro	2	470	
			Inert	0		
Champion-International, SC: Loblolly pine						
Weeds and small trees for several years, soil shaping in 1978	10-10-10	1,120	MyBr	67		March 1979
	lime	1,120	Chloro	33	392	
	trace		Inert	0		
	elements	34				
Hiwassee, GA: Loblolly pine						
Sorghum in 1976 and 1978, pine seedlings in 1977; 2.5-cm layer of sawdust disked in September 1978	20-20-20	560	MyBr	98		April 1979
	lime	1,120	Chloro	2	381	
			Inert	0		
Vallonia, IN: Virginia pine						
Hardwood seedlings in 1976 and 1978, sorghum-sudan grass in 1977	12-12-12	448	MyBr	98		May 1979
			Chloro	2	560	
			Inert	0		

<sup>1</sup> Fertilizers were applied just before bed shaping and study installation.

<sup>2</sup> MyBr—methyl bromide; Chloro—chloropicrin.

APPENDIX VIII. Chemical ( $\mu\text{g/g}$ ) and physical characteristics of soil at installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1979.

Nursery location and tree species	Total N	Avail- able P	Exchangeable			Per- cent organic matter	pH	Per- cent sand	Per- cent silt	Per- cent clay	Soil type
			K	Ca	Mg						
IMRD, GA:											
Loblolly pine	296	18	52	196	29	2.4	5.1	86	6	8	loamy sand
Buckeye, FL:											
Slash pine	335	60	30	162	21	0.7	5.7	88	5	7	loamy sand
International, MS:											
Loblolly pine	916	37	69	1,046	164	1.2	6.3	15	62	23	silt loam
Champion, SC:											
Loblolly pine	289	26	12	176	31	0.7	6.0	86	6	8	loamy sand
Hiwassee, GA:											
Loblolly pine	787	47	55	397	36	1.3	5.6	51	37	12	loam
Vallonia, IN											
Virginia pine	408	101	63	131	18	0.7	5.1	81	9	10	loamy sand

APPENDIX IX. Fertilizers and pesticides added during nursery tests of vegetative inoculum of *Pisolithus tinctorius* begun in 1979.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides		
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha	No. appl.
IMRD, GA: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	168	3	None used			None used			dimethoate	3.0	11
Buckeye, FL: Slash pine											
10-10-10	112	1	bifenox	5.6 l	2	ferbam	3.4	48	dicofol	4.6	1
NH <sub>4</sub> NO <sub>3</sub>	57	2	mineral spirits	187 l	2						
International Paper, MS: Loblolly pine											
13-13-13	112	1	glyphosate	4.5 kg	2	None used			diazinon	1.1	1
Fe chelate	2.2	1	bifenox	2.8 kg	2						
NH <sub>4</sub> NO <sub>3</sub>	178	5									
KCl	112	1									
Champion-International, SC: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	100	5	glyphosate	4 l	1	ferbam	2.2	17	None used		
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	112	1	bifenox	5.6 l	3						
KCl	112	1	nitrofen	11.2 l	2						
Hiwassee, GA: Loblolly pine											
NH <sub>4</sub> NO <sub>3</sub>	140	2	mineral spirits	187 l	4	fenaminosulf	22	1	None used		
Fe chelate	3.4	1	bifenox	3.4 kg	1	captan	28	1			
Vallonia, IN: Virginia pine											
12-12-12	448	2	None used			benomyl	1.1	3	None used		

APPENDIX X. Cropping history, fertilizers and fumigants used prior to installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1980.

Three-year cropping history	Fertilizers <sup>1</sup>		Fumigants			
	Type	kg/ha	Percent composition <sup>2</sup>		kg/ha	Date
Buckeye, FL: Slash pine						
Pine seedlings in 1977 and 1978, peas (summer) and rye grass (winter) in 1979	10-10-10	784	MyBr	68.4	570	March 1980
			Chloro	1.6		
			Inert	30.0		
Champion-International, SC: Loblolly pine						
Soybeans in 1977, fallow in 1978 and pine seedlings in 1979	10-10-10	960	MyBr	98	448	March 1980
	lime	1,120	Chloro	2		
	trace		Inert	0		
	elements	34				
Westvaco, SC: Loblolly pine						
Mixed pine-hardwoods till 1978, soil shaping in 1979	10-10-10	1,120	MyBr	98	403	April 1980
	lime	1,120	Chloro	2		
			Inert	0		

<sup>1</sup> Fertilizers were applied just before bed shaping and study installation.

<sup>2</sup> MyBr—methyl bromide; Chloro—chloropicrin.

APPENDIX XI. Chemical ( $\mu\text{g/g}$ ) and physical characteristics of soil at installation of nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1980.

Nursery location and tree species	Total N	Avail-able P	Exchangeable			Per-cent organic matter	pH	Per-cent sand	Per-cent silt	Per-cent clay	Soil type
			K	Ca	Mg						
IMRD, GA:											
Loblolly pine	305	23	56	210	37	2.2	5.1	87	5	8	loamy sand
Buckeye, FL:											
Slash pine	268	49	25	91	11	0.4	4.7	88	6	6	loamy sand
Champion, SC:											
Loblolly pine	260	31	49	56	7	0.4	5.1	88	6	6	loamy sand
Westvaco, SC:											
Loblolly pine	517	13	49	199	59	1.1	5.3	86	7	7	loamy sand

APPENDIX XII. Fertilizers and pesticides added during nursery tests of vegetative inoculum of *Pisolithus tinctorius* in 1980.

Fertilizers			Herbicides			Fungicides			Insecticides or nematicides	
Type	kg/ha	No. appl.	Name	Rate/ha	No. appl.	Name	kg/ha	No. appl.	Name	kg/ha
IMRD, GA: Loblolly pine										
NH <sub>4</sub> NO <sub>3</sub>	168	4	None used			None used			None used	
Buckeye, FL: Slash pine										
NH <sub>4</sub> NO <sub>3</sub>	56	2	bifenox	4.5 l	2	ferbam	3.4	38	None used	
KCl	28	2	mineral spirits	187 l	1					
Champion-International, SC: Loblolly pine										
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	100	5	bifenox	5.6 l	2	triadimefon	1.1	3	None used	
K <sub>2</sub> SO <sub>4</sub>	78	1								
KCl	112	1								
NH <sub>4</sub> NO <sub>3</sub>	112	1								
Westvaco, SC: Loblolly pine										
NH <sub>4</sub> NO <sub>3</sub>	112	1	bifenox	2.8 kg	2	ferbam	2.8	24	None used	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	112	1	nitrofen	9.3 l	12					
KCl	112	1								